8L680

October 27, 1998
Translated from the
Japanese Report

Sponsor

Nippon Zeon Co., Ltd.

REPORT

Acute Inhalation Study of Octafluorocyclopentene for 1 hour in Rats

(Study Number : 8L680)

October 14, 1998

Mitsubishi Chemical Safety Institute Ltd.

Statement

Kashima Laboratory

Mitsubishi Chemical Safety Institute Ltd.

Sponsor : Nippon Zeon Co., Ltd.

Title : Acute Inhalation Study of Octafluorocyclopentene for 1 hour in Rats

Study Number : 8L680

The study described in this report was conducted in compliance with the following GLP.

OECD principles of Good Laboratory Practice (1997)

October 14, 1998

Manager Osamu Fujii Sealed

Quality Assurance Statement

Kashima Laboratory

Mitsubishi Chemical Safety Institute Ltd.

Sponsor : Nippon Zeon Co., Ltd.

Title : Acute Inhalation Study of Octafluorocyclopentene for 1 hour in Rats

Study Number : 8L680

The study described in this report was conducted in compliance with the protocol and standard operation procedures. The methods and procedures reported herein are an accurate description of those employed in this study. The results presented in this report form a true and accurate representation of the raw data.

Stages of study	Date of inspection	Date of reporting inspection findings
Protocol	September 7, 1998	September 7, 1998
Amendment of the protocol	September 22, 1998	September 22, 1998
Study procedure	September 22, 1998	September 22, 1998
Raw data and draft report	October 11, 1998	October 12, 1998
Final report	October 14, 1998	October 14, 1998

October 14, 1998

Quality Assurance Manager Kunihiko Ofuchi Sealed

Quality Assurance Staff Mikiko Suzuki Sealed

Outline of the study

1. Title : Acute Inhalation Study of Octafluorocyclopentene for 1 hour in Rats
(Study Number : 8L680)

2. Purpose : The acute inhalation toxicity of Octafluorocyclopentene is assessed by exposing rats for 1 hour.

3. Method : Testing methods and evaluation for risk assessment of substance (Maritime Technology and Safety Bureau, Ministry of Transport, 1990)

4. GLP : OECD Principles of Good Laboratory Practice (1997)

5. Sponsor : Nippon Zeon Co., Ltd.
6-1 Marunouchi 2-chome, Chiyoda-ku, Tokyo, Japan
Responsible Person Kuniaki Goto

6. Organization under contract :
Mitsubishi Chemical Safety Institute Ltd.
1-30 Shiba 2-chome, Minato-ku, Tokyo, Japan

7. Testing facility : Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd.
14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, Japan

8. Responsible personnel :

Study Director	October 14, 1998	Hideaki Hiratsuka	Sealed

9809-61 Doaihoncho 4-chome, Hasaki-machi, Kashima-gun, Ibaraki, Japan

Study Staff	October 14, 1998	Toshiaki Sanada	Sealed

9. Study schedule : Initiation of the study September 7, 1998

Receipt of test animals	September 16, 1998
Administration	September 22, 1998
Necropsy	October 6, 1998
Issue of the final report	October 14, 1998

10. Environmental factors which were effected on the quality of the study : None

11. Archives : The protocol, specimens, all raw data, documents, and the final report will be retained in the archives of the Kashima Laboratory. Specimens and raw data, however, will be retained for the period of 5 years after submission of the final report, after which time the sponsor will be contacted to determine the disposition of these materials.

Contents

Summary

The acute inhalation toxicity of Octafluorocyclopentene (OFCPE) was assessed by exposing groups of 5 male and 5 female SD (SPF) rats to OFCPE gas for 1 hour.

Exposure was conducted using a whole body exposure chamber (approximately 95 L internal volume). Target concentrations of OFCPE in the chambers were 250, 500, 1000, 2000 and 4000 ppm; chemical analysis of gas samples taken from the test chambers showed the actual concentrations to be 210, 493, 1127, 1941 and 4233 ppm, respectively. Achieved concentrations were thus close to target concentrations; they were also confirmed to be stable during the exposure period.

Three males and one female exposed at 1000 ppm died after exposure, as did all males and females exposed at 2000 and 4000 ppm. Rats in the 1000, 2000 and 4000 ppm groups showed decreased locomotor activity, crouching and/or prone position, tonic convulsion (episthotonus), irregular respiration, dyspnea, hypothermia, nasal discharge, and smudge of the perinasal area.

Body weights in the 250 and 500 ppm groups increased normally. Body weights of surviving males and females in the 1000 ppm group were suppressed or decreased on Day 4, but increased normally thereafter.

Macroscopic examination of decedent rats revealed incomplete contraction, congestion and/or edema, plus petechial hemorrhage of the lung, atrophy of the spleen and distention of the stomach (filled with food). Macroscopic examination of surviving rats at the end of the observation period revealed no test substance-related abnormal findings.

The LC_{50} for OFCPE under the conditions of study was estimated to be 980 ppm for males, 1290 ppm for females, and 1124 ppm for the combined sexes.

Materials and methods

1. Test substance

Octafluorocyclopentene (OFCPE, CAS no. 559-40-0, Lot no. 980903, purity>99.99%) supplied by Nippon Zeon Co., Ltd. was used. The test substance is a liquid, and its chemical name, molecular formula, molecular weight, and boiling point are described below.

Data confirming stability of the test substance were received from the sponsor.

Chemical name	: 1,2,3,3,4,4,5,5-octafluorocyclopentene
Molecular formula	: C_5F_8
Molecular weight	: 212.04
Boiling point	: 27℃.

2. Test animal

Thirty one male and female CD (Sprague-Dawley) rats (SPF) were obtained from Charles River Japan, Inc. on September 16, 1998.

After acclimatization for 6 days, all rats were confirmed to be in good health. One day before administration, the rats were assigned to groups so that the mean body weight of each group was approximately the same (achieved by use of a stratified randomization procedure based on body weight). On the day of administration, rats were 5 weeks old, and body weights ranged from 150 to 167 g for males and from 130 to 143 g for females.

Rats were identified by ear punch (showing group number) and tailmarking (showing animal number). A label showing study number, name of test substance, animal number, sex, exposure concentration, date of administration, animal species and strain was attached to each cage.

3. Animal management

Throughout the study period, including the acclimatization and quarantine periods, the animal room was maintained automatically at a temperature of 22±2℃ (target range) and a relative humidity of 55±15% (target range). Ventilation comprised 12 changes per hour and the lighting cycle was 12 hours per day (7:00-19:00).

Within each group, the 5 rats of the same sex were housed together in a polycarbonate cage (265W×426D×200Hmm, Tokiwa Kagaku Kikai Co., Ltd.) with hard wood chip bedding (Betachip : Charles River Japan, Inc.); cages were placed on a steel rack. Sterilized stainless steel feeders for pelletted chow (Tokiwa Kagaku Kikai Co., Ltd.) and sterilized polycarbonate water bottles (700 mL, Tokiwa Kagaku Kikai Co., Ltd.) were used. The cages, together with the bedding, feeder, and water bottle, were changed weekly.

Rats were allowed free access to a pelletted experimental animal chow (MF : Oriental Yeast Co., Ltd.) and ordinary tap water (filtered through a 5 μm filter and UV-irradiated). However, the chow and water were withheld during exposure period. The chow and water were changed weekly.

Contaminant levels in the bedding and chow, such as pesticide residues, were confirmed to be within the acceptable range for our laboratory. The water was analyzed periodically and the results were confirmed to be within the specifications of the Waterworks Law in Japan.

4. Administration

Inhalation exposure was selected as the administration route to assess the risks associated with possible human exposure by inhalation. Rats were individually held for exposure in wire-mesh cages which were placed in a whole body exposure chamber (approximately 95 L internal volume). Animals were thus exposed for 1 hour.

Because the 4-hour LC_{50} of OFCPE was known to be approximately 500 ppm, the 1-hour LC_{50} of OFCPE was expected to be in the region of 1000 ppm. Consequently, exposure concentrations were set at 250, 500, 1000, 2000 and 4000 ppm.

5. Inhalation exposure system

5.1 Generation and exposure (Figure 1)

The test substance was bubbled with nitrogen gas and vaporized. The vaporized test substance was mixed with clean air, then supplied to the chamber using a one-pass method. Exposure commenced when the relative mass concentration (described below) became stable.

5.2 Analysis of exposure concentration

Gas in the chamber was collected with a gas-tight syringe (Dynatech Precision Sampling Corporation) at the start and end of exposure. The collected gas was analyzed as follows, and the exposure concentration was calculated. The change of gas concentration in the chamber was monitored by continuous measurement of the relative mass concentration of exhaust gas using a hydrocarbon meter (HCM-1B : Shimadzu Corporation).

・Method of analysis

Analysis apparatus	: Gas-chromatograph (GC), GC-14B, Shimadzu Corporation
Detector	: FID
Column	: G-100 (Chemicals Inspection and Testing Institute)
Column Temp.	: 150℃
Injection Temp.	: 170℃
Detector Temp.	: 170℃
Carrier gas	: Helium (20 mL/min)
Gas volume injected	: 1 mL

5.3 Test chamber environment

Temperature and relative humidity in the chamber were measured using a digital thermo-humidity meter (FCTH-990, Tokyo Glass Corporation) at the start and end of exposure.

6. Observations and measurements

6.1 Clinical observations

On the day of administration, the rats were observed for clinical signs just before exposure, just after exposure, plus 1 and 2 hours after exposure (4 times in total). Thereafter, the rats were observed for clinical signs once a day for 14 days.

6.2 Body weights

The day of administration was designated as Day 1. The body weights of all surviving rats were measured immediately prior to exposure, then on Days 4, 8, and 15 using an electric balance (EB-3200S, Shimadzu Corporation). In addition, the body weights of decedents were measured at the time death was discovered.

6.3 Pathological examination

1) Macroscopic examination

All the dead rats and surviving rats (Day 15) were necropsied. Rats found dead were necropsied immediately. The surviving rats were sacrificed (on Day 15) by exsanguination from the abdominal aorta under anesthesia with sodium thiopental (Ravonal : Tanabe Seiyaku Co., Ltd.) and subjected to necropsy.

2) Storage of organs

Because macroscopic examination revealed test substance-related abnormal findings in the lung, lungs and main organs (heart, trachea, liver, kidneys and spleen) removed from representative dead rats (more than 2 rats of each sex) and surviving rats (2 rats of each sex) were fixed in 10 % neutral phosphate-buffered formalin.

Results and conclusion

1. Exposure concentration (Figure 2, Table 1)

Target concentrations of OFCPE in the test chambers were 250, 500, 1000, 2000 and 4000 ppm; and chemical analysis of gas samples taken from the chambers found 210, 493, 1127, 1941 and 4233 ppm, respectively. Achieved concentrations were thus close to target concentrations; they were also confirmed to be stable during the exposure period.

2. Test chamber environment (Table 2)

The temperature in the chambers ranged from 24.3 to 25.3 ℃, and the relative humidity ranged from 40 to 59 %.

3. Mortality and LC_{50} (Table 3)

Three males and one female exposed at 1000 ppm died after exposure, as did all males and females exposed at 2000 and 4000 ppm. LC_{50} values (calculated from the mortality using Van der Waerden method) were estimated to be 980 ppm (95 % confidence interval 829-1160 ppm) for males, 1290 ppm (95 % confidence interval 1124-1479 ppm) for females, and 1124 ppm (95 % confidence interval 1005-1258 ppm) for the combined sexes.

4. Clinical signs (Table 4, Appendix 1)

Rats in the 1000, 2000, and 4000 ppm groups showed decreased locomotor activity, crouching and/or prone position, tonic convulsion (episthotonus), irregular respiration, dyspnea, hypothermia, nasal discharge, and smudge of the perinasal area.

5. Body weights (Figure 3, Table 5, Appendix 2)

Body weights in the 250 and 500 ppm groups increased normally. Body weights of surviving males and females in the 1000 ppm group were suppressed or decreased on Day 4, but increased normally thereafter.

6. Necropsy findings (Table 6, Appendix 3)

Macroscopic examination of dead rats revealed incomplete contraction, congestion and/or edema and petechial hemorrhage of the lung, atrophy of the spleen and distention of the stomach (filled with food). At the end of the observation period, dilatation of the pelvis (bilateral) was observed in one female in the 500 ppm group, however, this finding was considered incidental and not treatment-related.

7. Conclusion

The LC_{50} for OFCPE under the conditions of study was estimated to be 980 ppm for males, 1290 ppm for females, and 1124 ppm for the combined sexes.

Figures and Tables

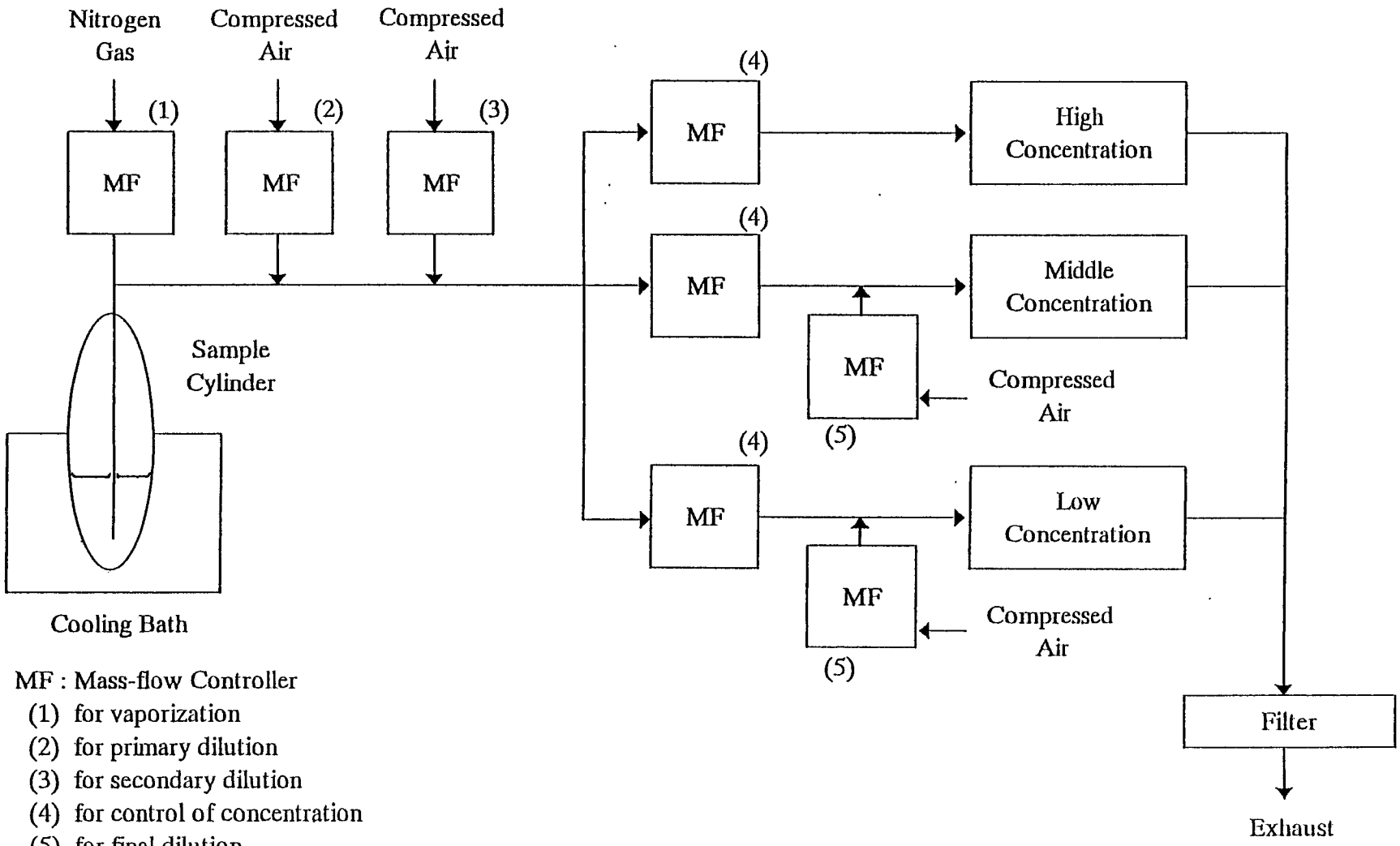
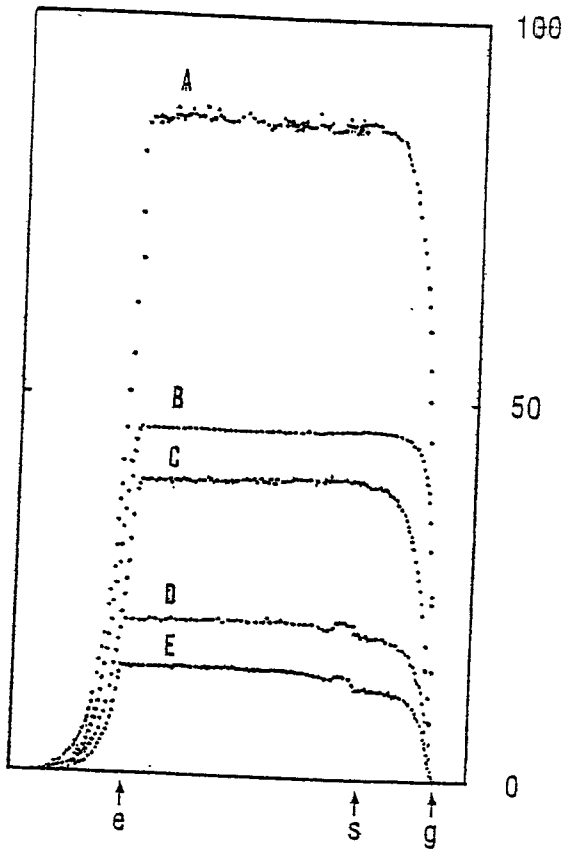


Figure 1 Flow-Chart of Inhalation Exposure System



A : 4000 ppm group
B : 2000 ppm group
C : 1000 ppm group
D : 500 ppm group
E : 250 ppm group

g : Start of Gas Generation
s : Start of Exposure
e : End of Exposure

Figure 2 Relative Mass Concentration

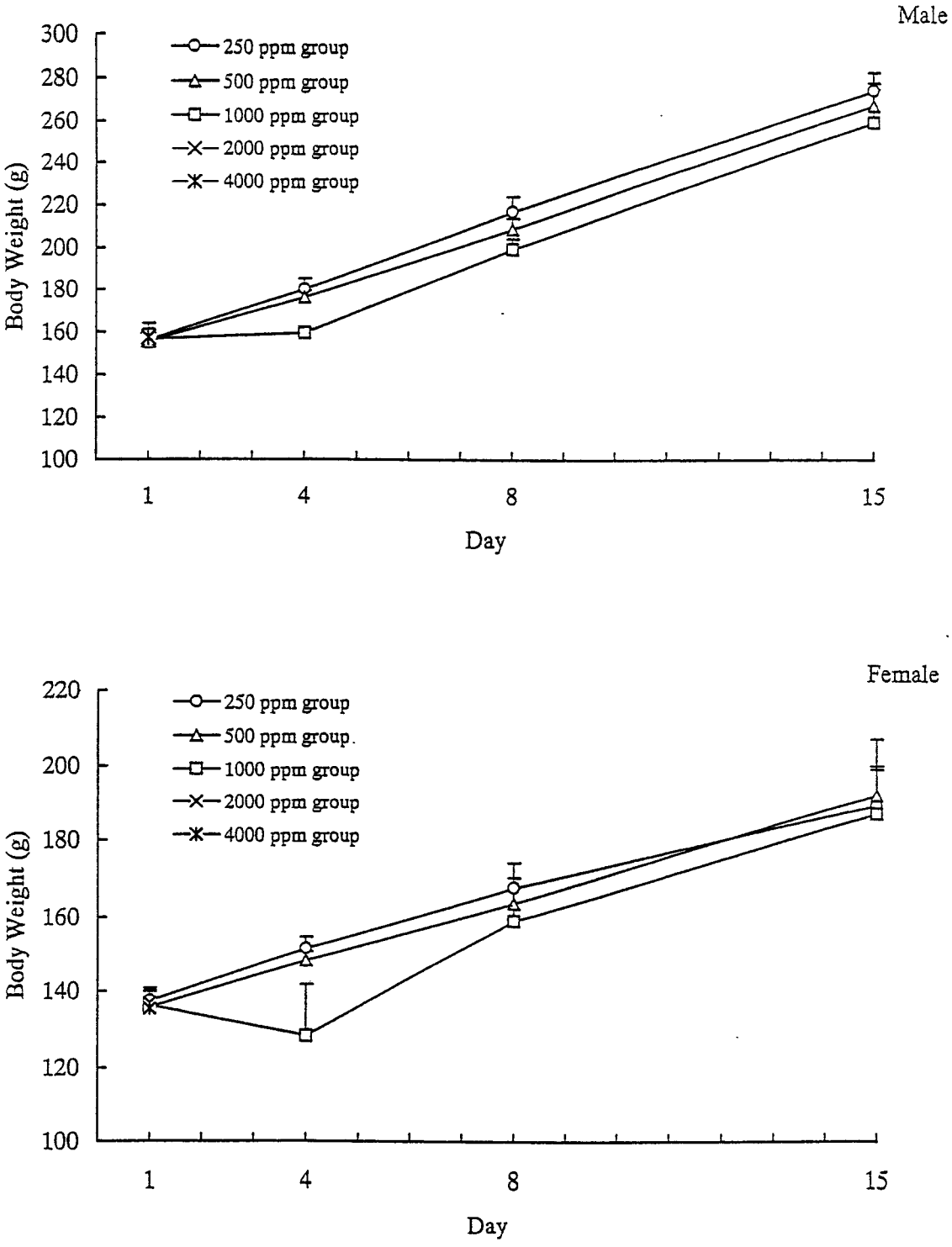


Figure 3 Body Weight

Table 1 Exposure Concentration

(ppm)

Target Concentration	Start of Exposure	End of Exposure	Mean Concentration
250	201	218	210
500	486	499	493
1000	1105	1149	1127
2000	1942	1940	1941
4000	4247	4218	4233

Table 2 Environment in Inhalation Chamber

Group	Item	Start of Exposure	End of Exposure
250 ppm group	Temp. (℃)	25.3	24.6
	R.H. (%)	41	42
500 ppm group	Temp. (℃)	25.3	25.0
	R.H. (%)	40	40
1000 ppm group	Temp. (℃)	24.3	24.6
	R.H. (%)	45	56
2000 ppm group	Temp. (℃)	24.5	24.7
	R.H. (%)	51	55
4000 ppm group	Temp. (℃)	24.9	25.0
	R.H. (%)	59	53

Temp. : Temperature, R.H. : Relative Humidity

Table 3 Mortality and LC_{50}

Target conc. (ppm)	Mean conc. (ppm)	Male	Female	Total
250	210	0/5	0/5	0/10
500	493	0/5	0/5	0/10
1000	1127	3/5	1/5	4/10
2000	1941	5/5	5/5	10/10
4000	4233	5/5	5/5	10/10
	LC_{50} (ppm)	980	1290	1124
	95% confidence interval (ppm)	829 - 1160	1124 - 1479	1005 - 1258

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats — Study No. 8L680

Table 4 Clinical Sign - Summary — Male

Test Substance Dose(ppm)	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
OFCPE	Number of Animals		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
250	No Abnormality		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
OFCPE	Number of Animals		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
500	No Abnormality		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
OFCPE	Number of Animals		5	5	5	5	5	4	2	2	2	2	2	2	2	2	2	2	2	2
1000	No Abnormality		5	0	0	0	4	2	2	2	2	2	2	2	2	2	2	2	2	2
	Death	+	0	0	0	0	1	2												
	Prone position	+	0	1	1	1	0	0												
	Crouching position	+	0	1	1	1	1	0												
	Decrease in	1	0	4	3	3	0	0												
	locomotor activity	2	0	1	1	0	0	0												
		3	0	0	1	2	1	0												
	Irregular respiration	+	0	2	2	1	1	0												
	Nasal discharge	1	0	1	0	0	0	0												
	Smudge of perinasal area	+	0	2	0	0	0	0												
OFCPE	Number of Animals		5	5	4	3	3													
2000	No Abnormality		5	0	0	0	0													
	Death	+	0	1	1	0	3													
	Prone position	+	0	1	2	2	0													
	Crouching position	+	0	3	1	1	1													
	Decrease in	2	0	3	0	0	0													
	locomotor activity	3	0	1	3	3	1													
	Tonic convulsion	+	0	0	0	2	0													
	Irregular respiration	+	0	3	2	2	1													
	Dyspnea	+	0	1	1	1	0													
	Hypothermia	+	0	0	0	0	1													
	Smudge of perinasal area	+	0	2	1	1	0													
)FCPE	Number of Animals		5	5																
4000	No Abnormality		5	0																
	Death	+	0	5																

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

cute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

able 4 Clinical Sign - Summary

Study No. 8L680

Female

Test Substance Dose(ppm)	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
OFCPE	Number of Animals		5	5	5	5	5	5	5	6	5	5	5	5	5	5	5	5	5	5
250	No Abnormality		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
OFCPE	Number of Animals		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
500	No Abnormality		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
OFCPE	Number of Animals		5	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4	4
1000	No Abnormality		5	0	0	0	3	2	4	4	4	4	4	4	4	4	4	4	4	4
	Death	+	0	0	0	0	0	1												
	Prone position	+	0	1	1	1	0	0												
	Crouching position	+	0	0	1	2	2	1												
	Decrease in	1	0	4	2	2	1	2												
	locomotor activity	2	0	1	2	2	1	0												
		3	0	0	1	1	0	0												
	Irregular respiration	+	0	2	3	2	1	0												
	Dyspnea	+	0	0	0	1	0	0												
OFCPE	Number of Animals		5	5	4	1	1	1												
2000	No Abnormality		5	0	0	0	0	0												
	Death	+	0	1	3	0	0	1												
	Prone position	+	0	2	0	0	0	0												
	Crouching position	+	0	0	1	1	1	0												
	Decrease in	2	0	1	0	0	1	0												
	locomotor activity	3	0	3	1	1	0	0												
	Tonic convulsion	+	0	2	0	0	0	0												
	Irregular respiration	+	0	2	1	1	1	0												
	Dyspnea	+	0	2	0	0	0	0												
	Smudge of perinasal area	+	0	2	0	0	0	0												
OFCPE	Number of Animals		5	5																
4000	No Abnormality		5	0																
	Death	+	0	5																

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Table 5 Body Weight - Summary — Male — Study No. 8L680 — Unit : g

Test Substance Dose (ppm)	Day	1	4	8	15
OFCPE 250	Mean	156.4	180.0	217.0	274.8
	S.D.	4.2	5.2	7.1	8.3
	n	5	5	5	5
OFCPE 500	Mean	155.6	176.2	209.0	268.0
	S.D.	3.6	3.4	4.9	10.4
	n	5	5	5	5
OFCPE 1000	Mean	156.6	159.5	199.5	260.0
	S.D.	4.6	2.1	4.9	15.6
	n	5	2	2	2
OFCPE 2000	Mean	156.2			
	S.D.	3.3			
	n	5			
OFCPE 4000	Mean	157.4			
	S.D.	6.5			
	n	5			

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Table 5 Body Weight - Summary — Female — Study No. 8L680 — Unit : g

Test Substance Dose(ppm)	Day	1	4	8	15
OFCPE 250	Mean	137.4	151.4	167.8	189.2
	S.D.	2.7	3.2	6.4	10.8
	n	5	5	5	5
OFCPE 500	Mean	135.8	148.2	163.8	192.0
	S.D.	5.0	2.5	6.6	15.2
	n	5	5	5	5
OFCPE 1000	Mean	136.2	128.3	159.0	187.0
	S.D.	3.6	13.4	8.8	12.1
	n	5	4	4	4
OFCPE 2000	Mean	136.8			
	S.D.	3.3			
	n	5			
OFCPE 4000	Mean	135.4			
	S.D.	4.4			
	n	5			

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Table 6 Necropsy Findings - Summary

Study No. 8L680

Organ / Findings	Sex	Male				
	Test Substance	OFCPE	OFCPE	OFCPE	OFCPE	OFCPE
	Dose (ppm)	250	500	1000	2000	4000
	Number of Animals	5	5	5	5	5
	Number of Animals Examined	〈5〉	〈5〉	〈5〉	〈5〉	〈5〉
Lung						
Congestion		0	0	3	4	3
Edema		0	0	1	3	2
Hemorrhage		0	0	0	1	1
Incomplete contraction		0	0	0	1	1
Stomach						
Distention		0	0	1	0	0

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Table 6 Necropsy Findings - Summary

Study No. 8L680

Organ / Findings	Sex	Female				
	Test Substance	OFCPE	OFCPE	OFCPE	OFCPE	OFCPE
	Dose (ppm)	250	500	1000	2000	4000
	Number of Animals	5	5	5	5	5
	Number of Animals Examined	〈5〉	〈5〉	〈5〉	〈5〉	〈5〉
Spleen						
Atrophy		0	0	1	0	0
Lung						
Congestion		0	0	1	2	4
Edema		0	0	0	0	4
Stomach						
Distention		0	0	1	0	0
Kidney						
Dilatation, pelvis		0	1	0	0	0

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 1- 1 Clinical Sign OFCPE 250 ppm Male Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10101	No Abnormality																			
10102	No Abnormality																			
10103	No Abnormality																			
10104	No Abnormality																			
10105	No Abnormality																			

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;

Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats — Study No. 8L680

Appendix 1- 2 Clinical Sign — OFCPE — 500 ppm — Male

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10201	No Abnormality																			
10202	No Abnormality																			
10203	No Abnormality																			
10204	No Abnormality																			
10205	No Abnormality																			

f , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats — Study No. 8L680

Appendix 1- 3 Clinical Sign — OFCPE — 1000 ppm — Male

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10301	Decrease in locomotor activity			1	1	1														
	Nasal discharge			1																
	Smudge of perinasal area			+																
10302	Death						+													
	Prone position			+	+	+														
	Crouching position						+													
	Decrease in locomotor activity			2	3	3	3													
	Irregular respiration			+	+	+	+													
10303	Death							+												
	Decrease in locomotor activity			1	1	1														
10304	Crouching position				+	+														
	Decrease in locomotor activity			1	2	3														
	Irregular respiration			+	+															
10305	Death							+												
	Crouching position			+																
	Decrease in locomotor activity			1	1	1														
	Smudge of perinasal area			+																

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OPCPE for 1 Hour in Rats — Study No. 8L680

Appendix 1- 4 Clinical Sign — OPCPE — 2000 ppm — Male

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10401	Death						+													
	Prone position			+																
	Crouching position				+	+	+													
	Decrease in locomotor activity			3	3	3	3													
	Tonic convulsion					+														
	Irregular respiration						+													
	Dyspnea			+	+	+														
	Hypothermia						+													
10402	Death			+																
10403	Death				+															
	Crouching position			+																
	Decrease in locomotor activity			2																
	Irregular respiration			+																
	Smudge of perinasal area			+																
10404	Death						+													
	Prone position				+	+														
	Crouching position			+																
	Decrease in locomotor activity			2	3	3														
	Irregular respiration			+	+	+														
	Smudge of perinasal area			+	+	+														

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 1- 4 Clinical Sign OFCPE 2000 ppm Male Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10405	Death						†													
	Prone position				†	†														
	Crouching position			†																
	Decrease in locomotor activity			2	3	3														
	Tonic convulsion					†														
	Irregular respiration			†	†	†														

†, Present; 1, Slight; 2, Moderate; 3, Severe;
Time 10, Before exposure; Time 20, Just after exposure; Time 30, 1 hr after exposure; Time 40, 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 1- 5 Clinical Sign OFCPE 4000 ppm Male Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
10501	Death			†																
10502	Death			†																
10503	Death			†																
10504	Death			†																
10505	Death			†																

†, Present; 1, Slight; 2, Moderate; 3, Severe;
Time 10, Before exposure; Time 20, Just after exposure; Time 30, 1 hr after exposure; Time 40, 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 1- 6 Clinical Sign OFCPE 250 ppm Female Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
50101	No Abnormality																			
50102	No Abnormality																			
50103	No Abnormality																			
50104	No Abnormality																			
50105	No Abnormality																			

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;

Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 1- 7 Clinical Sign — OFCPE — 500 ppm — Female — Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
50201	No Abnormality																			
50202	No Abnormality																			
50203	No Abnormality																			
50204	No Abnormality																			
50205	No Abnormality																			

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats — Study No. 8L680

Appendix 1- 8 Clinical Sign — OFCPE — 1000 ppm — Female

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
50301	Prone position				+	+														
	Crouching position						+	+												
	Decrease in locomotor activity			1	3	3	2	1												
	Irregular respiration			+	+		+													
	Dyspnea					+														
50302	Decrease in locomotor activity			1	1	1														
50303	Decrease in locomotor activity			1	1	1														
50304	Prone position			+																
	Crouching position				+	+	+													
	Decrease in locomotor activity			2	2	2	1	1												
	Irregular respiration				+	+														
50305	Death							+												
	Crouching position					+														
	Decrease in locomotor activity			1	2	2														
	Irregular respiration			+	+	+														

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
50401	Death				+															
	Prone position			+																
	Decrease in locomotor activity			3																
	Dyspnea			+																
	Smudge of perinasal area			+																
50402	Death			+																
50403	Death							+												
	Crouching position				+	+	+													
	Decrease in locomotor activity			2	3	3	2													
	Irregular respiration			+	+	+	+													
	Smudge of perinasal area			+																
50404	Death				+															
	Decrease in locomotor activity			3																
	Tonic convulsion			+																
	Irregular respiration			+																
50405	Death				+															
	Prone position			+																
	Decrease in locomotor activity			3																
	Tonic convulsion			+																
	Dyspnea			+																

+ , Present; 1 , Slight; 2 , Moderate; 3 , Severe;
Time 10 , Before exposure; Time 20 , Just after exposure; Time 30 , 1 hr after exposure; Time 40 , 2 hrs after exposure;

cute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

ppendix 1-10 Clinical Sign OFCPE 4000 ppm Female Study No. 8L680

Animal Number	Findings	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Time	10	20	30	40														
50501	Death			†																
50502	Death			†																
50503	Death			†																
50504	Death			†																
50505	Death			†																

†, Present; 1, Slight; 2, Moderate; 3, Severe;
Time 10, Before exposure; Time 20, Just after exposure; Time 30, 1 hr after exposure; Time 40, 2 hrs after exposure;

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 1 Body Weight OFCPE 250 ppm Male Study No. 8L680 Unit : g

Day / Animal Number	1	4	8	15
10101	160	186	222	281
10102	150	174	206	263
10103	160	185	224	281
10104	157	178	218	280
10105	155	177	215	269

Acute Inhalation Toxicity Study of OPCPE for 1 Hour in Rats

Appendix 2- 2 Body Weight OPCPE 500 ppm Male Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
10201	151	171	201	254
10202	161	178	210	267
10203	156	175	208	263
10204	156	180	213	280
10205	154	177	213	276

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 3 Body Weight OFCPE 1000 ppm Male Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
10301	154	161	203	271
10302	156	...	...	...
10303	164	...	...	...
10304	152	158	196	249
10305	157	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 4 Body Weight OFCPE 2000 ppm Male Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
10401	152	...	...	...
10402	159	...	...	...
10403	156	...	...	...
10404	160	...	...	...
10405	154	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 5 Body Weight OFCPE 4000 ppm Male Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
10501	150	...	...	...
10502	160	...	...	...
10503	167	...	...	...
10504	156	...	...	...
10505	154	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 6 Body Weight OFCPE 250 ppm Female Study No. 8L680 Unit : g

Day / Animal Number	1	4	8	15
50101	140	155	173	191
50102	139	153	176	205
50103	134	148	161	175
50104	135	148	164	186
50105	139	153	165	189

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 7 Body Weight OFCPE 500 ppm Female Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
50201	133	149	161	191
50202	135	148	163	200
50203	130	144	156	170
50204	138	150	165	188
50205	143	150	174	211

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 8 Body Weight OFCPE 1000 ppm Female Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
50301	132	109	147	176
50302	139	140	165	197
50303	133	132	158	177
50304	140	132	166	198
50305	137	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 2- 9 Body Weight OFCPE 2000 ppm Female Study No. 8L680

Unit : g

Day / Animal Number	1	4	8	15
50401	133	...	...	...
50402	142	...	...	...
50403	137	...	...	...
50404	137	...	...	...
50405	135	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Study No. 8L680

Appendix 2-10 Body Weight OFCPE 4000 ppm Female Unit : g

Day / Animal Number	1	4	8	15
50501	134	...	...	...
50502	133	...	...	...
50503	135	...	...	...
50504	143	...	...	...
50505	132	...	...	...

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 3 Necropsy Findings Death or Moribund Sacrifice Study No. 8L680

		Male				Female			
Sex	:								
Test Substance	:	OFCPE	OFCPE	OFCPE		OFCPE	OFCPE	OFCPE	
Dose (ppm)	:	1000	2000	4000		1000	2000	4000	
Animal No.	:	1 1 1	1 1 1 1 1	1 1 1 1 1		5	5 5 5 5 5	5 5 5 5 5	
		0 0 0	0 0 0 0 0	0 0 0 0 0		0	0 0 0 0 0	0 0 0 0 0	
		3 3 3	4 4 4 4 4	5 5 5 5 5		3	4 4 4 4 4	5 5 5 5 5	
		0 0 0	0 0 0 0 0	0 0 0 0 0		0	0 0 0 0 0	0 0 0 0 0	
		2 3 5	1 2 3 4 5	1 2 3 4 5		5	1 2 3 4 5	1 2 3 4 5	
Organ Findings	Abnormality :	Y Y Y	Y Y Y Y Y	Y Y N Y Y		Y	N Y Y N N	Y N Y Y Y	
Spleen									
Atrophy						+			
Lung									
Congestion		+ + +	+ +	+ +	+ +	+	+ +	+	+ + +
Edema		+	+	+ +	+	+		+	+ + +
Hemorrhage, focal			+		+				
Incomplete contraction			+		+				
Stomach									
Distention		+				+			

+ . Present

N . Finding absent; Y . Finding present

Acute Inhalation Toxicity Study of OFCPE for 1 Hour in Rats

Appendix 3 Necropsy Findings Scheduled Sacrifice (Week 3) Study No. 8L680

		Male										Female									
Sex	:																				
Test Substance	:	OFCPE					OFCPE					OFCPE					OFCPE				
Dose (ppm)	:	250					500					1000					250				
Animal No.	:	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		1	1	1	1	1	2	2	2	2	2	3	3	1	1	1	1	1	2	2	2	3	3
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		1	2	3	4	5	1	2	3	4	5	1	4	1	2	3	4	5	1	2	3	4	5
Organ Findings	Abnormality	: N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
Kidney																							
Dilatation, pelvis																			†				
																			B				

†, Present; B, Bilateral

N, Finding absent; Y, Finding present

Statement of English Translation

Study Title	: Acute Inhalation Study of Octafluorocyclopentene for 1 hour in Rats
Study Number	: 8L680
Study Director	: Hideaki Hiratsuka
Type of the document	: Report

I hereby confirm that this English report is an exact translation of the original Japanese report on the study which was conducted in Mitsubishi Chemical Safety Institute Ltd.

Translated by : Hideaki Hiratsuka Date : October 27, 1998

Hideaki Hiratsuka, Ph.D.

Kashima Laboratory

Mitsubishi Chemical Safety Institute Ltd.

OFCPE

ACUTE INHALATION (4 HOUR) STUDY IN RATS

Sponsor

Nippon Zeon Co Ltd,
Furukawa Sogo Bldg,
6-1 Marunouchi 2-chome,
Chiyoda-ku,
Tokyo 100,
JAPAN.

Research Laboratory

Huntingdon Life Sciences Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued: 16 January 1998

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by Nippon Zeon Co Ltd. These results may not be published, either wholly or in part, or reviewed or quoted in any other publication without the prior authorisation of the Sponsors.

WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989, and subsequently the United Kingdom Good Laboratory Practice Regulations 1997.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Minsitry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Derek W Coombs, B.Sc.,
Study Director,
Huntingdon Life Sciences Ltd.

16 January 1998

Date

CONFIDENTIAL BUSINESS INFORMATION

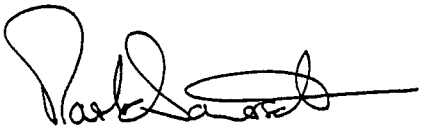
ZCE 13/972861

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Certain studies such as that described in this report, are conducted at Huntingdon in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Management, Huntingdon Life Sciences.

Date(s) of inspection	16 & 18 June 1997
Date(s) of reporting inspection findings to the Study Director and Management	20 June 1997
Date of reporting audit findings to the Study Director and Management.	11 September 1997



15 January 1998

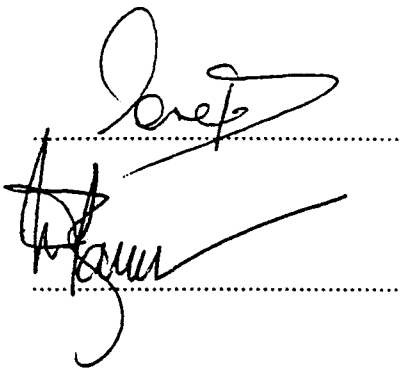
Mark Somerset,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

Date

RESPONSIBLE PERSONNEL

STUDY MANAGEMENT

Derek W. Coombs, B.Sc.,
Study Director.



Mario Bannerman, H.N.D.,
Head of Inhalation Toxicology.

CONTENTS

SUMMARY

Test substance

A low boiling point liquid identified as OFCPE.

Test animals

Albino rats, (Sprague-Dawley). One control group and 4 test groups each of 5 male and 5 female rats.

Route of administration

By inhalation of a test atmosphere containing a vapour generated from the test substance.

Duration of exposure

4 hours continuous snout only exposure.

Observation period

14 days post exposure.

Results

Exposure levels and mortality

Group	Level (mg/l)	Mortality		
		M	F	Total
1	Control	0/5	0/5	0/10
2	10233 ppm	5/5	5/5	10/10
3	960 ppm	5/5	5/5	10/10
4	188 ppm	0/5	0/5	0/10
5	430 ppm	2/5	1/5	3/10

All rats exposed to OFCPE at 10233ppm died within 50 minutes of the start of exposure.

One male rat exposed at 960 ppm died during the exposure, the remaining rats exposed at this level were dead by Day 1 of the observation period.

For rats exposed to OFCPE at 430 ppm one male and one female were found dead on Day 2 of the observation period. The other male was found dead during Day 3 of the observation period.

Clinical signs

During exposure signs seen in rats exposed to OFCPE at 10233 ppm were struggling in the restraint tube, reduced respiration rate, irregular respiration, slow laboured breathing followed by death.

In rats exposed at 960 ppm signs seen during exposure included struggling in the restraint tube (initially), diaphragmatic (pronounced breathing), red ears, convulsions (1 rat only), deep respiration and rapid respiration. One male exposed at this level died in the restraint tube.

For rats exposed at 430 ppm of OFCPE struggling in the restraint tube and exaggerated breathing were seen during exposure.

During exposure to OFCPE at 188 ppm the only clinical sign seen was red ears.

Signs seen in rats immediately after exposure to OFCPE at 960 ppm were exaggerated respiratory movements, intermittent whole body tremors, lethargy, pilo erection, hypothermia of the whole body, discharge from the eyes (red/clear), eyes partially closed, wet fur on the snout and jaws, brown staining around the snout and/or jaws. All rats were dead by Day 1 of the observation period.

Signs seen in rats following exposure to OFCPE at 430 ppm were exaggerated respiratory movements, pilo erection, emaciation and eyes partially closed. Signs seen in females only exposed at this level were ataxia, sensitivity to sound, brown staining around the snout and/or jaws and yellow staining around the uro-genital region. Surviving males and 2 females exposed at this level were normal in appearance and behaviour by Days 5 and 8 of the observation period respectively. The other 2 surviving females were sensitive to sound up to and including Day 14 of the observation period.

In rats exposed at 188 ppm red/brown staining around the eyes was seen in 3/5 males and 3/5 females. All rats exposed at this level were normal in appearance and behaviour by Day 1 of the observation period.

Fur soiled with excreta was seen in some rats during and immediately following exposure. This sign is attributed to the method of restraint.

Bodyweight

All rats surviving exposure to OFCPE initially lost weight. Rats exposed at 188 or 430 ppm had a similar rate of bodyweight gain to the control rats by Days 2 and 4 of the observation period respectively.

Food and water consumption

Food consumption in rats surviving exposure to OFCPE at 188 or 430 ppm was markedly reduced for 2 or 4 days, respectively. Otherwise, food consumption for surviving test rats was similar to that of the controls.

Water consumption in surviving rats exposed to OFCPE at 430 ppm was reduced for 4 days following exposure to OFCPE. Males exposed to OFCPE at 188 ppm had a reduced water consumption for 1 day. Otherwise water consumption for surviving test rats was similar to that of the controls.

Macroscopic pathology

An external abnormality seen in rats exposed at 10233 ppm was wet fur on the snout and jaws.

External abnormalities seen in decedent rats exposed at 960 ppm were matted fur, brown staining (snout/jaws/eyes/uro-genital region), wet fur on the snout and jaws (slightly brown for 1 rat) and wet fur. Brown staining around the snout was noted in one male exposed at 430 ppm.

Fur soiled with excreta was noted in some decedent rats. This is attributed to the method of restraint.

Congestion of the lungs (patchy/moderate/severe) was seen in rats exposed at 10233, 960 or 430 ppm. Frothy (white) fluid in the trachea was seen in all rats exposed at 10233 or 960 ppm.

In addition a fluid filled stomach and a blood filled chest cavity was seen in a single rat exposed at 960 ppm OFCPE.

Further abnormalities seen in rats surviving exposure at 430 ppm included pale subpleural areas on all lobes of the lungs, dark subpleural foci on the lungs, gas filled stomach, congested stomach, thickened stomach (glandular region), stomach light in colour (glandular region), congested small intestine, red fluid filled small intestine, small and dark spleen and dark areas on the liver.

For rats exposed at 188 ppm no macroscopic abnormalities were noted.

Dark areas on the lungs (1 male) and liver (1 female) were seen in 2 control rats. These findings are considered to be incidental and of no toxicological significance.

CONCLUSION

The LC_{50} (4 hour) for OFCPE is estimated at 459 ppm in air for males and females combined.

INTRODUCTION

The acute inhalation toxicity of OFCPE was assessed by exposing 4 groups of rats, for a period of 4 hours, to an atmosphere produced from the test substance at concentrations of 10233, 960, 430 or 188 ppm of air. A further group, acting as a control was exposed to clean air only.

The study was conducted at Huntingdon Life Sciences during the period 2 April and 22 May 1997.

The protocol for the study was approved by the Study Director and HRC Management on 18 March 1997 and approved by the Sponsor on 25 March 1997.

The study design was in compliance with EEC and OECD test guidelines for acute inhalation studies.

On completion of the study all data relating to the study, including a copy of the final report, were lodged in the Huntingdon Life Sciences Archives, Huntingdon, Cambridgeshire, England.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

TEST SUBSTANCE

Identity:	OFCPE (1, 2, 3, 3, 4, 4, 5, 5 Octafluorocyclopentene)
Intended use:	Raw material for the production of HFCPA
Lot no.:	9703-1
Appearance:	Liquid
Storage:	4°C in the original container
Purity:	>99.7%
Amount received:	1590 g
Expiry:	Assumed to be stable for the duration of the study
Date received:	25 March 1997
Supplier:	Nippon Zeon Co Ltd

The full description of the chemical and physical properties of the test substance are the responsibility of the Sponsor.

A small sample (1 - 2 g) was sealed in a suitable container and stored in Archives at an appropriate temperature.

MATERIALS AND METHODS

ANIMALS AND MAINTENANCE

Twenty five male and 25 female albino rats (Sprague-Dawley), were selected from three consignments of rats obtained from Charles River UK Ltd, Manston Road, Margate, Kent, England on 2, 18 and 30 April 1997. The rats were selected so that males and females would be approximately 7 weeks and 8 weeks old on the day of delivery to Huntingdon Life Sciences.

On arrival the rats were allocated to 1 of 5 groups, each of 5 males and 5 females and were identified individually by a number tattooed on the ears or on the right hind foot to indicate 100's. The rats were housed by sex in groups of 5 and acclimatised to laboratory conditions for at least 5 days before the day of exposure.

The holding cages (size 35 cm × 53 cm × 25 cm height) were made of stainless steel sheet and wire mesh and were suspended on a movable rack. While in their cages all rats had free access to a measured excess amount of food (SDS RM1) and tap water. Food and water supplies were analysed routinely to determine the levels of chemical or microbiological contaminants. Room lighting was by artificial light between 8 am and 8 pm daily.

The rats remained in a holding room except for the 4-hour exposure periods and an overnight post exposure period when the rats in the test groups were kept in a ventilated cabinet to allow dispersal of any residual test substance.

The temperature and relative humidity of the holding room air was monitored continuously using a Kent Clearspan thermohygrograph. The temperature of the holding area during the study remained within the range of 21°C± 3°C and the relative humidity generally remained within the range 55% ± 15%. There were no extremes of temperature or humidity considered likely to have influenced the results of the study.

INHALATION EXPOSURES

Four groups of rats were exposed continuously for 4 hours to a test atmosphere containing the vapour of the test substance.

A further group acting as a control received clean air only for 4 hours.

The group identifications and dates of exposure for the groups were:

Group 1 (Control):	15 April 1997
Group 2 (Test):	15 April 1997
Group 3 (Test):	24 April 1997
Group 4 (Test):	25 April 1997
Group 5 (Test):	8 May 1997

The mean concentrations of the test atmospheres for Groups 2 to 5 are given in the **RESULTS** section of this report.

EXPOSURE SYSTEM

Atmosphere generator

The atmosphere generator, shown in Figure 1, was designed to produce and maintain an atmosphere containing vapour by evaporation of the test substance from a fritted glass disc with a countercurrent of air. All parts of the generator in contact with the test substance were made of glass. The test substance was delivered to the generator at a constant flow rate directly from the bulk cylinder of OFCPE by a micro metering valve, or as liquid from polypropylene syringes mounted on an infusion pump, connected by PTFE tubing (Group 5 only). The air supplied to the generator was dried, filtered and oil free.

Exposure chambers

The snout-only exposure chambers were of cylindrical form and made of aluminium alloy. The chambers used for Groups 1, 2 and 3 (10 cm diameter and 65 cm height) had an enclosed volume of approximately 5 litres, those used for Groups 4 and 5 (30 cm diameter and 45 cm height) had an enclosed volume of approximately 30 litres. The rats were held for exposure in moulded polycarbonate tubes which were attached at evenly spaced ports in the cylindrical section of the chamber. The tubes were tapered at one end to allow the snout only to project into the chamber. The other end was closed by insertion of an expanded plastic bung. A push rod passed through the centre of the bung and was adjusted to maintain the position of a rat during exposure

The test atmosphere entered the chamber through a port at the top centre of the chamber and was extracted at the base centre below the level of the rats. Each chamber was installed in a large fume cupboard exhausting through an absolute filter.

PROCEDURE

A supply of clean dried air was connected to the vapour generator and the supply pressure was adjusted to give a flow rate of 2 litres per minute measured at the generator outlet tube. An in-line flow meter was used to monitor air flow throughout the exposure.

For Groups 2, 3 and 4 the bulk cylinder of OFCPE was connected to the generator with PTFE tubing. For Group 5 a sample of the test substance was placed in a syringe and connected to the generator with PTFE tubing. The generator was situated in a water bath maintained at 20 - 25°C. The flow rate selected for the first exposure was expected to give a vapour concentration of approximately 10,000 ppm.

The rats to be exposed were placed into restraining tubes. The tubes were attached to the ports in the mid section of the chamber.

The exposure was timed for 4 hours, following a 6-minute, (Group 2), or 2 minute (Groups 3, 4 and 5) equilibration period [1].

After 4 hours, the supply of test substance was discontinued and the exposure chamber was allowed to clear before the rats were removed for examination.

[1] The theoretical time required for the concentration of vapour in the chamber to reach 90% of its final value under the conditions of exposure employed

The bulk cylinder/syringe weight of OFCPE was recorded at the start and finish of each exposure.

This procedure was repeated for Groups 3, 4 and 5 using air flows of 10 litres per minute for Group 3 and 30 litres per minute for Groups 4 and 5. Different flow rates were employed in the study in order to minimise usage of the test substance, which was in a limited supply. The different flow rates used were considered not to have affected the integrity of the study.

Following exposure, the rats were returned to the holding cages and food and water supplies were restored. The test rats were kept in a ventilated cabinet overnight and then returned to the holding room for the remainder of the observation period.

The control group was treated similarly but exposed to clean dried air only.

The control rats were returned to the holding room at the end of the exposure procedure.

CHAMBER ATMOSPHERE ANALYSES

Between 5 and 11 air samples (dependant on the length of exposure and the repeatability of samples) were taken from the chamber during each exposure. The concentration of OFCPE in the chamber air was determined by chemical analysis.

Each air sample was withdrawn into a gas tight syringe.

The method of chemical analysis is described in Appendix 1.

OBSERVATIONS

Clinical signs

The rats were observed continuously for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period. The clinical signs were recorded as they were observed during the exposure but in the case of tube restraint were severely limited. During the observation period, the clinical signs were recorded once in the morning and then as necessary following a later check for clinical signs.

Bodyweight

All rats were weighed daily from the day of delivery to the Huntingdon Life Sciences up to and including the day of sacrifice/death.

Food and water consumption

The amount of food and water consumed by each cage of rats was measured daily from the day of arrival to sacrifice/death. The daily mean intakes of food and water for each rat were calculated from the recorded data.

TERMINAL STUDIES

At the end of the 14-day observation period, the rats were killed by intraperitoneal injection of pentobarbitone sodium and exsanguinated.

All rats were subjected to a detailed macroscopic examination. Tissues were not retained.

ESTIMATION OF THE LC_{50} (4-HOUR) AND STANDARD ERROR

The concentration of the test substance likely to cause death in 50% of exposed rats following a single 4 hour exposure was calculated by the log probit method of Miller and Tainter [1].

The standard error and 95% confidence limits (95% CL) were calculated from the formulae:

$$\text{SE of } LC_{50} = \frac{2S}{\sqrt{2N}}$$

where 2s is the estimated increment in concentration of the test substance between probits 4.0 and 6.0 corresponding to 16% and 84% mortality and N is the total number of rats in groups with mortality between 6.7% and 93.3% (probits 3.5 - 6.5).

[1] Miller, L.C. and Tainter, M.L., *Proc. Soc. Exp. Bio. Med.* 57, (2), 1944, pp 261 - 264

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Concentration of OFCPE

The results of the chemical analysis for the air samples taken during the exposures are shown in Table 1.

The mean concentration of OFCPE were:

Group	Concentration of OFCPE in air (ppm)
2	10233
3	960
4	188
5	430

Chamber air temperature

The mean chamber air temperature and the standard deviation (sd) of the means during exposure of the groups were:

Group	Temperature (°C)	Standard deviation
1 (Control)	19	0.2
2 (10233 ppm)	20	0.0
3 (960 ppm)	20	0.4
4 (188 ppm)	21	0.3
5 (430 ppm)	21	0.0

There were no extremes of temperature considered likely to have influenced the results of the study.

CLINICAL OBSERVATIONS

Mortality

Group	Level (ppm)	Mortality		
		M	F	Total
1	Control	0/5	0/5	0/10
2	10233ppm	5/5	5/5	10/10
3	960 ppm	5/5	5/5	10/10
4	188 ppm	0/5	0/5	0/10
5	430 ppm	2/5	1/5	3/10

All rats exposed to OFCPE at 10233 ppm died within 50 minutes of the start of exposure.
One male rat exposed at 960 ppm died during the exposure, the remaining rats exposed at this level were dead by Day 1 of the observation period.

For rats exposed to OFCPE at 430 ppm one male and one female were found dead on Day 2 of the observation period. The other male was found dead during Day 3 of the observation period.

No deaths occurred as a result of exposure to 188 ppm OFCPE.

Clinical signs

During the exposure

Signs seen during exposure were severely restricted to those most obvious under the conditions of tube restraint.

During exposure signs seen in rats exposed to OFCPE at 10233ppm were struggling, a reduced respiration rate, irregular respiration, slow laboured breathing followed by death.

In rats exposed at 960 ppm signs seen during exposure included struggling (initially), diaphragmatic (pronounced breathing), red ears, convulsions (1 rat only), deep respiration and rapid respiration. One male exposed at this level died in the restraint tube.

For rats exposed at 430 ppm of OFCPE struggling and exaggerated breathing were seen during exposure.

During exposure to OFCPE at 188 ppm the only clinical sign seen was red ears.

During the observation period

The incidence of clinical signs seen during the observation period is shown in Table 2. Column 0 of this table shows the observations made when the rats were removed from the exposure chamber.

Signs seen in rats immediately after exposure to OFCPE at 960 ppm were exaggerated respiratory movements, intermittent whole body tremors, lethargy, pilo erection, hypothermia of the whole body, discharge from the eyes (red/clear), eyes partially closed, wet fur on the snout and jaws, brown staining around the snout and/or jaws and death.

Signs seen in rats following exposure to OFCPE at 430 ppm were exaggerated respiratory movements, pilo erection, emaciation and eyes partially closed. Signs seen in females only exposed at this level were ataxia, sensitivity to touch, brown staining around the snout and/or jaws and yellow staining around the uro-genital region. Surviving males and 2 females exposed at this level were normal in appearance and behaviour by Days 5 and 8 of the observation period respectively. The other 2 surviving females were sensitive to sound up to and including Day 14 of the observation period.

In rats exposed at 188 ppm red/brown staining around the eyes was seen in 3/5 males and 3/5 females. All rats exposed at this level were normal in appearance and behaviour by Day 1 of the observation period.

Fur soiled with excreta was seen in some rats during and immediately following exposure. This sign is attributed to the method of restraint.

Bodyweight

The group mean and individual bodyweights are shown in Table 3. The group mean bodyweights are also shown in Figure 2.

All rats surviving exposure to OFCPE initially lost weight. Rats exposed at 188 or 430 ppm had a similar rate of bodyweight gain to the control rats by Days 2 and 4 of the observation period respectively.

Food consumption

The food consumption data are presented in Table 4.

Food consumption in rats surviving exposure to OFCPE at 188 or 430 ppm was markedly reduced for 2 or 4 days, respectively. Otherwise, food consumption for surviving test rats was similar to that of the controls.

Water consumption

The water consumption data are presented in Table 5.

Water consumption in surviving rats exposed to OFCPE at 430 ppm was reduced for 4 days following exposure to OFCPE. Males exposed to OFCPE at 188 ppm had a reduced water consumption for 1 day. Otherwise water consumption for surviving test rats was similar to that of the controls.

TERMINAL STUDIES

Macroscopic pathology

The macroscopic pathological findings for individual rats are summarised in Table 6.

Wet fur on the snout and jaws was seen in rats exposed at 10233 ppm.

Decedent rats exposed at 960 ppm were noted to have matted fur, brown staining (snout/jaws/eyes/uro-genital region), wet fur on the snout and jaws (slightly brown for 1 rat) and wet fur. Brown staining around the snout was noted in one male exposed at 430 ppm.

Fur soiled with excreta was noted in some decedent rats. This is attributed to the method of restraint.

Congestion of the lungs (patchy/moderate/severe).was seen in rats exposed at 10233, 960 or 430 ppm. Frothy (white) fluid in the trachea was seen in all rats exposed at 10233 or 960 ppm.

A fluid filled stomach was seen in 2/5 males and 3/5 females exposed at 960 ppm. A blood filled chest cavity was seen in a single rat exposed at 960 ppm OFCPE.

Further abnormalities seen in rats exposed at 430 ppm were pale subpleural areas on all lobes of the lungs, dark subpleural foci on the lungs, gas filled stomach, congested stomach, thickened stomach (glandular region), stomach light in colour (glandular region), congested small intestine, red fluid filled small intestine, small and dark spleen and dark areas on the liver.

No macroscopic abnormalities were seen for rats exposed at 188 ppm OFCPE.

Dark areas on the lungs (1 male) and liver (1 female) were seen in 2 control rats. These findings are considered to be incidental and of no toxicological significance.

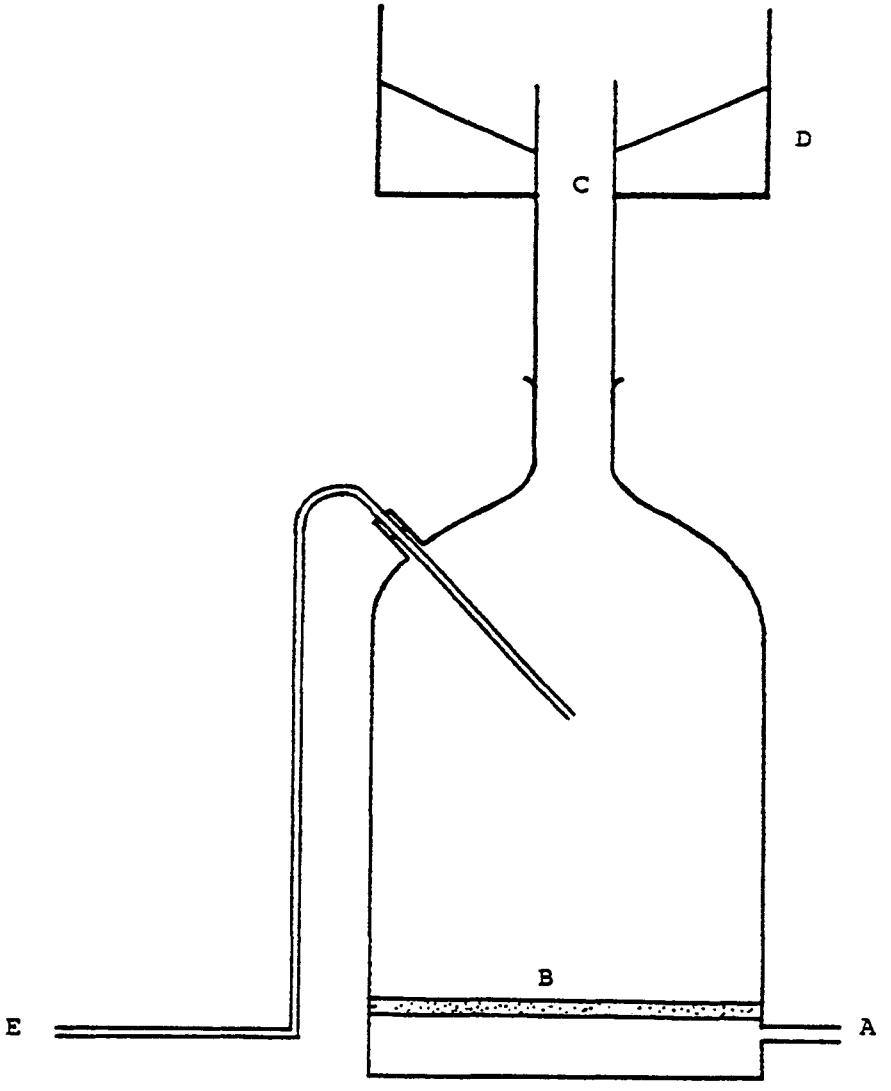
Estimation of the LC_{50} (4-hour) for OFCPE

From the mortality data for Groups 2, 3, 4 and 5, the LC_{50} (4 hour) for OFCPE and 95% confidence limits (95% CL) were established at:

	LC_{50} (4 hour) (ppm)	95% CL (ppm)
Males	445	159.9-729.6
Females	490	175.7-805.0
Combined	459	286.4-631.1

FIGURE 1

Vapour generator



A - Air inlet
B - Glass frit
C - Vapour outlet
D - Glass column connecting with the exposure chamber
E - Test liquid supply from infusion pump/cylinder

FIGURE 2

Group mean bodyweights - males

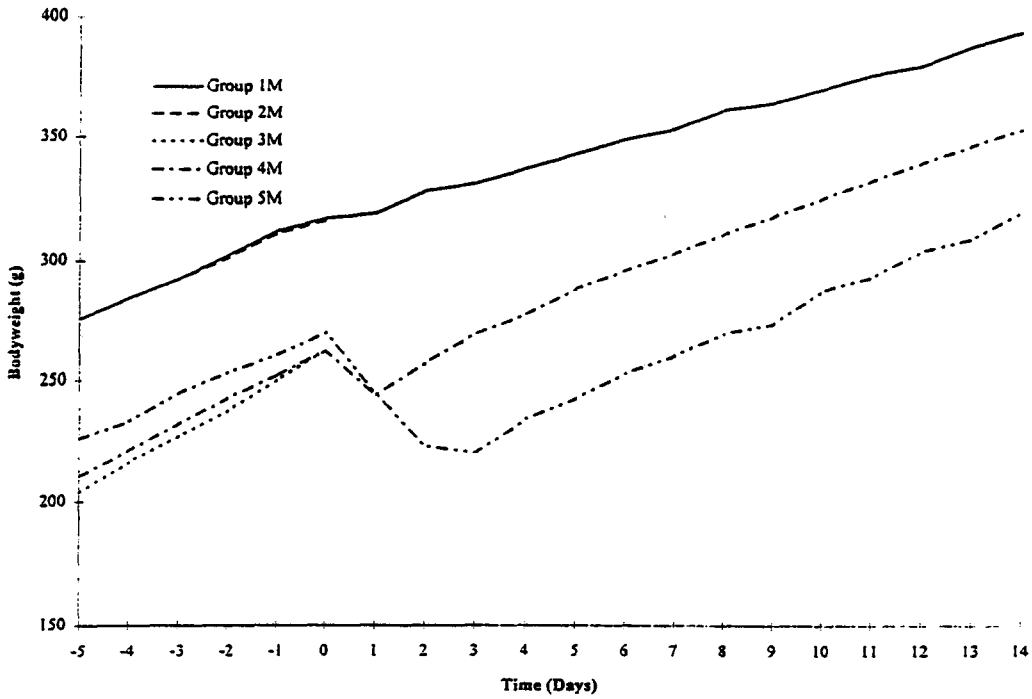


FIGURE 2

Group mean bodyweights - females

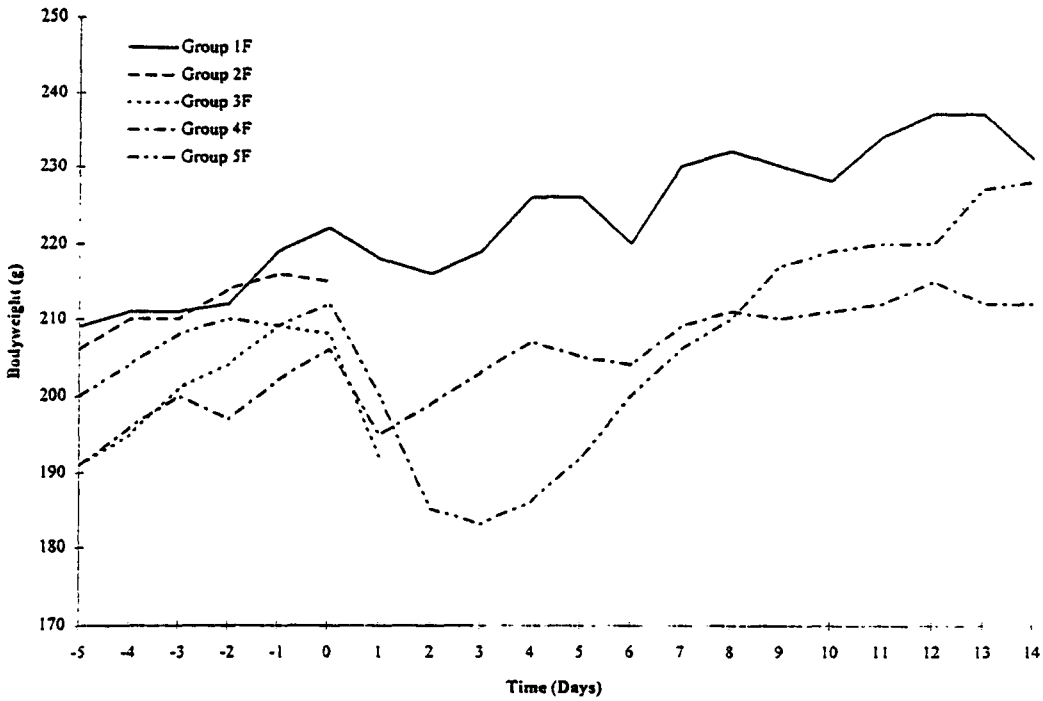


TABLE 1

Concentrations of OFCPE

Chemical analysis

Group	Sample	Time taken	Amount in air (ppm)
2	1	0h : 00m	*0
	2	0h : 10m	10475
	3	0h : 20m	10548
	4	0h : 30m	9678
	[a]5	0h : 51m	10231
	Time weighted mean		10233

* Note: Sampling/injection error. Value not included in the time weighted mean

[a] All rats were dead 50 minutes into exposure

TABLE 1

Concentrations of OFCPE

Chemical analysis

Group	Sample	Time taken	Amount in air (ppm)
3	1	0h : 00m	*961
	2	0h : 10m	941
	3	0h : 20m	923
	4	0h : 30m	962
	5	1h : 00m	1061
	5R		970
	6	2h : 00m	972
	7	3h : 00m	938
	8	4h : 00m	951
	Time weighted mean		960

* Note : This time zero result was not included in the time weighted mean
R Repeat sample

TABLE 1

Concentrations of OFCPE

Chemical analysis

Group	Sample	Time taken	Amount in air (ppm)
4 OFCPE	1	0h : 00m	*222
	2	0h : 10m	208
	3	0h : 20m	208
	4	0h : 30m	188
	5	1h : 00m	120
	5R		194
	6	2h : 00m	188
	7	3h : 00m	195
	8	4h : 00m	191
	Time weighted mean		188

* Note : This time zero result was not included in the time weighted mean
R Repeat sample

TABLE 1

Concentrations of OFCPE

Chemical analysis

Group	Sample	Time taken	Amount in air (ppm)
5	1	0h : 00m	*390
OFCPE	2	0h : 10m	411
	3	0h : 20m	459
	3R		465
	4	0h : 30m	468
	4R		414
	5	1h : 00m	431
	6	2h : 00m	435
	7	3h : 00m	407
	7R		424
	8	4h : 00m	433
	Time weighted mean		430

* Note: This time zero result was not included in the time weighted mean
R Repeat sample

TABLE 2

Clinical signs during observation period

Group	Signs	Number showing signs																	
		Day of observation period																	
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1M (Control)	Normal appearance and behaviour	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Wet fur	1	1																
1F (Control)	Normal appearance and behaviour	3	3	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Wet fur	2	2	1															

* Clinical signs recorded after exposure on the day of exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs															
		Day of observation period															
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2M (10223 ppm)	Dead (Total)	5[a]																
2F (10223 ppm)	Dead (Total)	5[a]																

* Clinical signs recorded after exposure on the day of exposure

[a] All rats died during exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs																
		Day of observation period																
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3M (960 ppm)	Fur soiled with excreta	4	3	3														
	Exaggerated respiratory movements	4	3	3														
	Eyes partially closed	3	2	2														
	Lethargic	3	2	1														
	Wet fur on the snout and jaws	3	2	2														
	Intermittent whole body tremors	4	3	3														
	Red discharge from the eyes	1	1	1														
	Dead (Total)	1	2	2	5													

* Clinical signs recorded after exposure on the day of exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs																
		Day of observation period																
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3F (960 ppm)	Fur soiled with excreta	5	5	5	1													
	Exaggerated respiratory movements	5	5	5	1													
	Eyes partially closed	2	1	1	1													
	Lethargic	4	3	2	1													
	Wet fur on the snout and jaws	2	2	1														
	Intermittent whole body tremors	4	5	5	1													
	Clear discharge from the eyes	3	1		1													
	Pilo erection		2	2	1													
	Hypothermia of the whole body				1													
	Brown staining around the snout and/or jaws			1	1													
	Dead (Total)				5													

* Clinical signs recorded after exposure on the day of exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs																
		Day of observation period																
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4M (188 ppm)	Normal appearance and behaviour			1	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur soiled with excreta	5	5	2														
	Red/brown staining around the eyes	3	3	3														
4F (188 ppm)	Normal appearance and behaviour			1	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur soiled with excreta	5	5	3														
	Red/brown staining around the eyes	3	3	3														

* Clinical signs recorded after exposure on the day of exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs																
		Day of observation period																
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5M (430 ppm)	Normal appearance and behaviour								3	3	3	3	3	3	3	3	3	3
	Fur soiled with excreta	5	5	5														
	Exaggerated respiratory movements	1	1	3	5	4	3	3										
	Eyes partially closed	1	1															
	Pilo erection			1	2	4	3											
	Emaciated				1													
	Dead (Total)					1	2	2	2	2	2	2	2	2	2	2	2	2

* Clinical signs recorded after exposure on the day of exposure

TABLE 2

(Clinical signs during observation period - continued)

Group	Signs	Number showing signs																
		Day of observation period																
		0hr*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5F (430 ppm)	Normal appearance and behaviour										1	2	2	2	2	2	2	2
	Fur soiled with excreta	5	5	5														
	Exaggerated respiratory movements	3	3	3	5	5	4	4	4	4	3							
	Eyes partially closed	3	3	1		1												
	Ataxia	5	5															
	Sensitive to touch				2				3	2	2	2	2	2	2	2	2	2
	Pilo erection					2												
	Brown staining around the snout and/or jaws				3													
	Yellow staining around the uro-genital region						2											
	Emaciated						1											
	Dead (Total)					1	1	1	1	1	1	1	1	1	1	1	1	1

* Clinical signs recorded after exposure on the day of exposure

TABLE 3

Individual and group mean bodyweights (g)

Group	Rat	Day of observation																			
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	101	281	292	301	310	324	331	338	346	352	359	367	374	374	383	386	395	401	408	417	420
	102	262	270	277	289	300	299	300	310	313	318	323	329	334	345	346	343	354	359	358	369
	103	265	273	282	292	302	304	308	314	319	327	335	337	340	343	347	355	355	362	368	376
	104	280	289	296	306	315	324	324	333	334	340	344	350	356	367	368	377	383	382	397	400
	105	285	294	302	313	320	329	325	335	339	343	348	355	361	369	375	378	389	390	398	403
	Mean	275	284	292	302	312	317	319	328	331	337	343	349	353	361	364	370	376	380	388	394
1F (Control)	106	219	220	224	219	233	236	232	228	234	244	241	234	247	258	250	243	258	258	260	252
	107	210	210	210	207	216	217	213	212	216	219	220	211	224	222	220	223	225	230	230	227
	108	194	200	200	203	203	209	207	204	202	211	208	207	208	210	214	215	212	218	219	214
	109	208	212	204	214	219	217	212	213	216	219	225	218	229	227	227	223	227	230	227	220
	110	213	215	219	215	225	229	224	222	228	236	234	229	241	241	241	236	248	248	249	242
	Mean	209	211	211	212	219	222	218	216	219	226	226	220	230	232	230	228	234	237	237	231

0 = Day of exposure

TABLE 3

(Individual and group mean bodyweights (g) - continued)

Group	Rat	Day of observation																		
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2M (10223 ppm)	111	281	292	300	311	320	327	Dead													
	112	256	261	271	278	285	290	Dead													
	113	277	286	295	306	314	320	Dead													
	114	300	314	321	333	348	351	Dead													
	115	260	266	275	279	290	292	Dead													
	Mean	275	284	292	301	311	316														
2F (10223 ppm)	116	214	208	219	219	225	218	Dead													
	117	191	204	205	208	202	207	Dead													
	118	214	225	224	226	225	231	Dead													
	119	205	209	200	209	214	210	Dead													
	120	206	205	202	210	213	209	Dead													
	Mean	206	210	210	214	216	215														

0 = Day of exposure

TABLE 3

Individual and group mean bodyweights (g)

Group	Rat	Day of observation																			
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3M	21	203	218	228	239	251	265	Dead													
(960 ppm)	22	198	208	221	230	243	251	Dead													
	23	205	216	227	233	248	259	Dead													
	24	208	221	228	242	250	262	Dead													
	25	206	224	234	245	257	271	Dead													
	Mean	204	217	228	238	250	262														
3F	26	192	196	201	201	201	207	Dead													
(960 ppm)	27	196	198	209	213	219	213	Dead													
	28	189	191	195	202	208	203	Dead													
	29	183	190	195	191	201	204	Dead													
	30	194	202	207	211	214	215	192	Dead												
	Mean	191	195	201	204	209	208	192													

0 = Day of exposure

TABLE 3

Individual and group mean bodyweights (g)

Group	Rat	Day of observation																			
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4M (188 ppm)	31	221	233	244	253	264	278	253	270	284	290	303	308	320	321	330	340	347	353	358	366
	32	212	221	233	244	252	260	245	250	263	277	285	293	302	312	323	327	339	350	355	359
	33	206	217	227	237	245	253	245	268	272	282	293	301	309	315	321	329	335	338	348	359
	34	210	217	230	236	244	254	229	235	254	257	269	278	283	290	293	303	308	318	323	328
	35	208	221	233	245	255	263	249	262	273	277	292	299	303	316	323	325	333	338	344	353
	Mean	211	222	233	243	252	262	244	257	269	277	288	296	303	311	318	325	332	339	346	353
4F (188 ppm)	36	183	189	193	192	192	199	188	189	195	196	193	193	197	197	198	194	198	200	196	196
	37	188	193	197	195	197	207	195	199	201	204	204	203	205	209	207	210	206	216	211	210
	38	196	204	201	197	202	209	193	205	205	210	211	204	214	216	218	214	218	217	218	212
	39	197	199	209	200	211	210	199	201	211	216	215	210	220	221	221	220	222	228	227	221
	40	191	194	198	199	206	204	200	200	205	208	204	209	211	210	205	215	214	216	209	219
	Mean	191	196	200	197	202	206	195	199	203	207	205	204	209	211	210	211	212	215	212	212

0 = Day of exposure

TABLE 3

Individual and group mean bodyweights (g)

Group	Rat	Day of observation																		
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5M (430 ppm)	91	236	244	256	265	273	284	259	Dead												
	92	237	244	253	258	266	275	257	237	227	245	253	265	272	277	285	291	298	311	314	325
	93	213	220	230	238	246	252	225	213	218	234	241	252	259	269	275	285	288	295	300	306
	94	224	228	238	250	255	263	238	214	Dead											
	95	226	235	247	254	261	269	247	232	219	227	234	242	250	260	269	284	293	306	313	328
	Mean	227	234	245	253	260	269	245	224	221	235	243	253	260	269	276	287	293	304	309	320
5F (430 ppm)	96	210	214	220	220	218	224	201	187	177	180	185	196	205	210	214	215	224	228	230	227
	97	203	205	208	213	217	217	209	191	188	200	207	217	228	236	244	248	244	239	250	252
	98	198	206	211	211	206	208	197	182	174	171	178	185	192	198	203	206	205	213	216	216
	99	199	196	201	203	205	204	198	180	Dead											
	100	192	198	202	201	199	205	196	184	192	194	199	202	199	195	205	208	207	200	211	215
	Mean	200	204	208	210	209	212	200	185	183	186	192	200	206	210	217	219	220	220	227	228

0 = Day of exposure

TABLE 4

Group mean daily food consumption (g/rat)

Group	Days																		
	Pre-exposure					Post-exposure													
	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	34	34	34	34	35	31	33	33	32	33	33	31	35	30	34	35	35	34	35
2M (10223 ppm)	32	33	34	34	34	Dead													
3M (960 ppm)	29	32	34	36	35	0	Dead												
4M (188 ppm)	31	34	33	33	33	13	25	31	32	34	35	34	32	35	33	36	35	37	37
5M (430 ppm)	29	29	31	31	31	8*	3*	12*	21*	31	28	29	32	33	33	32	34	33	36
1F (Control)	22	22	21	22	23	20	20	22	25	22	20	23	23	24	20	22	24	21	20
2F (10223 ppm)	24	21	24	23	23	Dead													
3F (960 ppm)	22	23	23	23	21	1*	Dead												
4F (188 ppm)	22	23	18	22	22	13	16	21	22	20	20	22	19	21	17	20	21	21	17
5F (430 ppm)	23	22	24	22	22	10*	2*	8*	13*	22	24	25	26	28	25	25	22	25	24

* Wet diet offered (diet and water mix) in an effort to induce appetite

TABLE 5

Group mean daily water consumption (g/rat)

Group	Days																		
	Pre-exposure					Post-exposure													
	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	33	33	33	36	32	33	33	33	33	32	32	33	36	31	32	35	34	31	34
2M (10223 ppm)	31	32	32	32	29	Dead													
3M (960 ppm)	33	35	36	38	35	Dead													
4M (188 ppm)	32	34	33	32	34	19	33	34	33	35	35	37	32	34	34	35	34	34	36
5M (430 ppm)	28	29	30	29	29	8	2	6	24	27	37	36	36	38	37	35	38	34	36
1F (Control)	28	26	26	29	29	28	22	28	30	28	25	28	28	31	24	27	29	26	26
2F (10223 ppm)	25	21	26	22	23	Dead													
3F (960 ppm)	26	28	26	27	24	4	Dead												
4F (188 ppm)	30	28	23	28	27	26	25	28	27	27	26	28	26	25	21	25	26	24	22
5F (430 ppm)	25	24	26	22	23	21	5	13	18	31	45	34	32	33	28	28	28	30	26

TABLE 6

Macroscopic pathology

Group	Rat	Region/organ affected	Observation
1M	101		No abnormalities detected
(Control)	102	Lungs	Dark areas (left lung, right posterior and right azygous lobes)
	103		No abnormalities detected
	104		No abnormalities detected
	105		No abnormalities detected
1F	106		No abnormalities detected
(Control)	107		No abnormalities detected
	108		No abnormalities detected
	109	Liver	Dark in colour (patchy)
	110		No abnormalities detected

TABLE 6

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
2M (10233 ppm)	111*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	112*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	113*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	114*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	115*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
2F (10233 ppm)	116*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	117*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	118*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	119*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid
	120*	External	Wet fur on the snout and jaws
		Lungs	Severely congested (all lobes)
		Trachea	Contained white frothy fluid

* Decedents

TABLE 6

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
3M (960 ppm)	21*	External	Matted fur, brown staining around the snout, jaws and eyes
		Lungs	Severely congested (patchy all lobes)
		Stomach	Fluid filled
	22*	External	Fur soiled with excreta, wet fur (slightly brown) on the snout and jaws
		Lungs	Congested (patchy all lobes)
		Trachea	Contained frothy fluid
	23*	External	Fur soiled with excreta, wet fur
		Chest cavity	Contained a large amount of blood
	24*	External	Matted fur
		Lungs	Severely congested (patchy all lobes)
	25*	External	Matted fur, brown staining around the snout and jaws
		Lungs	Severely congested (patchy all lobes)
		Stomach	Fluid filled
3F (960 ppm)	26*	External	Matted fur, brown staining around the uro-genital region
		Lungs	Severely congested (all lobes)
		Trachea	Contained frothy fluid
		Stomach	Fluid filled
	27*	External	Matted fur
		Lungs	Moderately congested (patchy all lobes)
	28*	External	Matted fur, brown staining around the snout and jaws
		Lungs	Severely congested (patchy all lobes)
		Trachea	Contained frothy fluid
		Stomach	Fluid filled
	29*	External	Matted fur, brown staining around the snout and jaws
		Lungs	Severely congested (patchy all lobes)
		Stomach	Fluid filled
	30*	External	Matted fur
		Lungs	Severely congested (patchy all lobes)
		Trachea	Contained frothy fluid

* Decedents

TABLE 6

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
4M (188 ppm)	31		No abnormalities detected
	32		No abnormalities detected
	33		No abnormalities detected
	34		No abnormalities detected
	35		No abnormalities detected
4F (188 ppm)	36		No abnormalities detected
	37		No abnormalities detected
	38		No abnormalities detected
	39		No abnormalities detected
	40		No abnormalities detected

TABLE 6

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
5M	91*	Lungs	Severely congested
(430 ppm)		Stomach	Glandular region congested and thickened
		Small intestine	Congested and contained red fluid
		Spleen	Small in size, dark in colour
		Liver	Dark in colour (patchy)
	92	Lungs	Pale subpleural areas (all lobes)
	93		No abnormalities detected
	94*	External	Brown staining around the snout
		Lungs	Severely congested
		Stomach	Congested
		Small intestine	Congested
		Liver	Dark in colour
	95	Lungs	Pale subpleural areas (all lobes)
5F	96		No abnormalities detected
(430 ppm)	97	Lungs	Pale subpleural areas (all lobes)
	98		No abnormalities detected
	99*	Lungs	Severely congested
		Stomach	Gas filled, glandular region light in colour
		Small intestine	Congested and contained red fluid
		Liver	Dark in colour (patchy)
	100	Lungs	Dark subpleural foci (all lobes)

* Decedents

APPENDIX 1

Compound specific inhalation analytical procedure for OFCPE

The analysis of OFCPE in air samples

This document details the basic procedure to be used for the GC assay of OFCPE in air samples at concentrations in the approximate range of 4,000 to 120,000 ppm. The samples are analysed by GC with FID detection.

Effective Date:	10 April 1997
Reference to Sponsor's methodology:	Yes (Fax dated 02/04/97)

Authorisation

The method outlined in this document has been validated and is considered fit for the purpose of monitoring chamber conditions in an Inhalation Toxicology study.

Prepared by:	**Approved by:**
Karen Boag	Ian S Gilkison
Analyst	Head of Section

This contains the core method for the analysis of OFCPE. Study specific amendments will be detailed on the study IAP cover sheet.

The signed original procedure is retained in the raw data.

APPENDIX 1

(Compound specific inhalation analytical procedure for OFCPE - continued)

Compound details

OFCPE	Octafluorocyclopentene. Clear liquid, storage at 4°C. Boiling point 27°C, Molecular weight 212g (C_5F_8).

Apparatus

Permanent items

Device	Make	Model
Gas sampling bags	Tedlar	1, 3 and 5L capacity
Metering Syringes	Hamilton Company	500ml;
	Dynatech	A2 250µl syringe with side port needle.
Gas sampling syringes	Dynatech	A2 100µl syringe with side port needle.
Balance	Sartorius	R160P with YDP-01 data print

Sampling of gas mixtures

Insert the needle of the 100 µl gas sampling syringe through the septum of the sampling port of the exposure chamber or standard gas bag. Open the syringe valve and flush the syringe with the sample twice by withdrawing and depressing the plunger of the syringe. Fill the syringe with gas sample and equilibrate pressure for 5 seconds. Close the syringe valve for transportation. Open the syringe valve, expel excess sample, allow to equilibrate, close the syringe valve. Put the injection needle in the GC sampling port. Open the syringe valve and expel the contents from the syringe. Press the run button.

GC injection.

30 µl of the chamber atmosphere / gas bag is injected onto the GC column. No split was used.

Preparation of standard gas bags

Evacuate the required number of gas bags (preferably 1,3 or 5 litres). Ensure they have been flushed prior to this and no air remains in the bag after evacuation. Using the appropriate syringe (500 ml metering syringe), transfer the required volume of room air into an evacuated gas bag via the side port valve. Transfer the gas gently to avoid leakage and heating effects. Inject each gas bag with the required volume of liquid OFCPE, ensuring the metering syringe (250 µl) used is weighed before and after the injection. To insure that all OFCPE is in the gaseous state, heat each gas bag with a hot air supply, for 1 minute and then allow to equilibrate back to room temperature. Thoroughly mix the gases by pressing on alternate sides several times.

APPENDIX 1

(Compound specific inhalation analytical procedure for OFCPE - continued)

The gas bag concentrations are calculated using the following equations

$$\text{Conc} = \frac{V}{V_a + V} \times 1{,}000{,}000 \text{ ppm and}$$

$$V = \frac{W \times R \times T}{M} \times \frac{760 \text{ mm Hg}}{Atm}$$

where V = gaseous volume of OFCPE (ml);
W = mass of OFCPE (mg);
M = molecular weight of OFCPE (212 mg/mmole);
R = 0.08205 ml.atm/mmol.K;
T = temperature (K);
Atm = atmospheric pressure (mmHg);
V_a = volume of air (ml).

Calibration procedure

Samples of the standard gas bags containing known amounts of OFCPE are injected onto the GC in duplicate. A response factor or gradient is calculated (by the PC1000 software) from the mean peak area for each standard.

Concentration = Area response/Gradient

Quality assurance measures.

Duplicate injections should not differ by more than 5%.

Analyse a standard gas mixture as a QA sample following every 6-8 samples. The relative error should be within 5% of the nominal value except at the limit of quantification, where 10% is acceptable.

On preparation of new standards the standard response factor should be checked (area response / concentration). The calibration standard will be compared against a second, independently prepared standard. The standard will be considered acceptable if the ratio of response factors is within the range 0.95 to 1.05.

If the above criteria are not met, review the data and system components for sources of error and repeat analysis or standard preparation as necessary.

APPENDIX 1

(Compound specific inhalation analytical procedure for OFCPE - continued)

Typical chromatographic conditions

Chromatograph	PU4550	Pye Unicam		
A/D interface	SP4500	Thermo Separation Products		
Integration software	PC1000	Thermo Separation Products		
Analytical column	DB-1, 5 µm film, 30 m x 0.53 mm i.d.			
Detector	Flame ionisation (range 10).			
Temperatures(°C)	Column	35°C		
	Injector	100°C		
	Detector	150°C		
Flow rates	Helium (carrier)		5	ml/min
	Detector make-up (He)		25	ml/min
	Hydrogen		30	ml/min
	Air		330	ml/min
Retention times	OFCPE	~ 1.8 minutes.		
Injection volume	30 µl.			
Analysis cycle	2.5 minutes.			

APPENDIX 2

Composition of OFCPE

CONFIDENTIAL 1/2

Composition of OFCPE

Lot. No. 9703 – 1

Analysis Date 1997. 2. 28

	Chemical Name	CAS No.	Content (%)
OFCPE	1, 2, 3, 3, 4, 4, 5, 5 – Octafluorocyclopentene	559 – 40 – 0	99.8213
Impurity – 1	1 – Chloro – 1, 2, 2, 3, 3, 4, 4, 5, 5 – nonafluorocyclopentane	376 – 76 – 1	0.0855
Impurity – 2	Chloro – heptafluorocyclopentene		0.0095
Impurity – 3	unknown		0.0178
Impurity – 4	unknown		0.0166
Impurity – 5	unknown		0.0162
Impurity – 6	unknown		0.0331
Impurity – 7	unknown		–
Impurity – 8	unknown		–
Impurity – 9	unknown		–
Total			100.000

APPENDIX 2 CONFIDENTIAL 2/2

(Composition of OFCPE - continued)
GLC Analysis

Sample Name	OFCPE
Lot. No.	9703 – 1
Analysis Date	1997. 2. 28

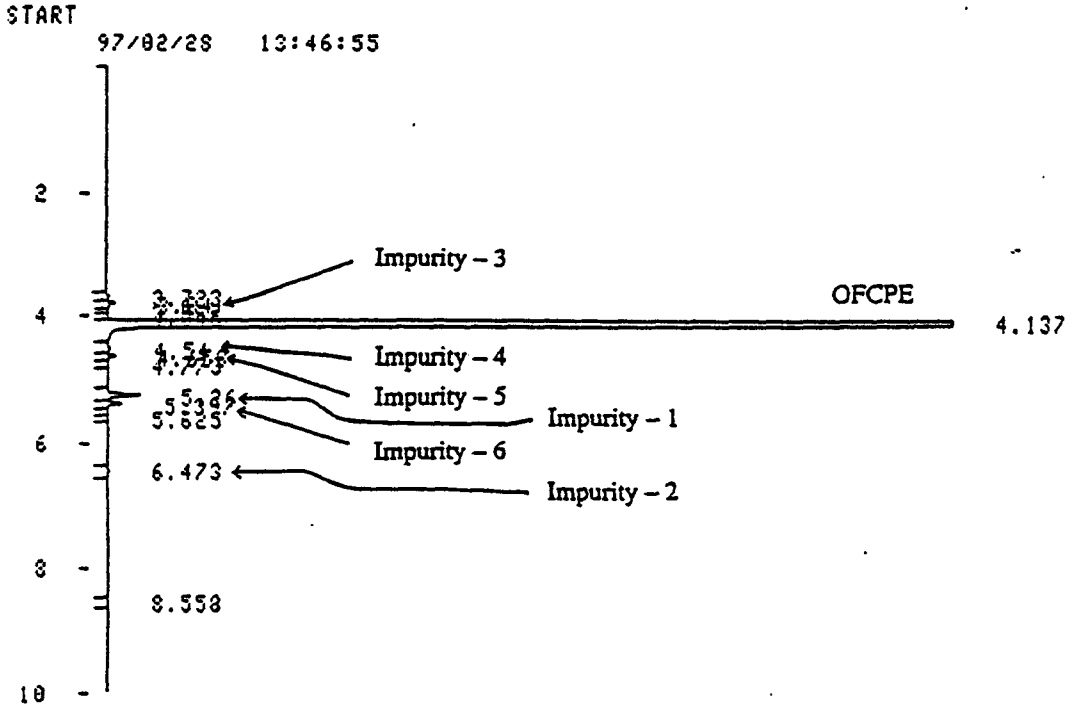
GLC

Apparatus	: Hitachi 263-70
Column	: Neutrabond-1 Capillary Column 50 m x ID 0.25 Φ (1.5 μ mdf)
Column Temp.	: 30 ℃
Inj. Temp.	: 150 ℃
Split Ratio	: 100
Carrier Gas	: N_2 (main : 100 ml/min., back up : 50 ml/min.)
Detector	: FID
Sample Size	: 1 μl

Integrator

Integrator	: Shimadzu C-R6A
Parameter	: Width 5, Slope 110, Drift 0, Min area 100, T-Dbl 0, Stp Tm 20, Atten 3, Speed 10, Method$ 461, Format$ 2001

Chart



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.84	205	V		0.0178	
2	4.137	1151837	V		99.8213	
3	4.54	191	V		0.0166	
4	4.658	187	V		0.0162	
5	5.26	986			0.0855	
6	5.397	382	V		0.0331	
7	6.473	110			0.0096	
				: 50 :		

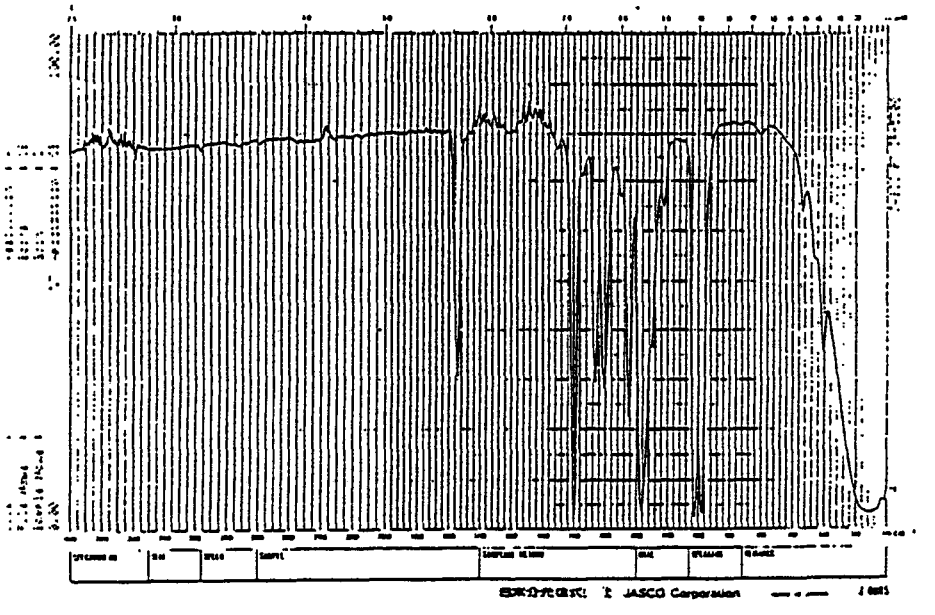
APPENDIX 2

(Composition of OFCPE - continued) CONFIDENTIAL

IR Data

Date	1996. 7. 12
Operator	Tetsuya Sugimoto (Organic Synthesis Lab., Corporate Reserch Lab., Nippon Zeon Co., Ltd.)
Sample Name	OFCPE
Lot. No.	9703 – 1
Condition	Gas, NaCl Gas Cell, room temp. (Apparatus: JASCO FT/IR – 5300)

Chart



ZCE 13/972861

APPENDIX 3

Protocol and Protocol Amendments

Huntingdon life Sciences Study Number: ZCE/13

CONFIDENTIAL

Huntingdon Life Sciences

PROTOCOL

OFCPE

ACUTE INHALATION (4 HOUR) STUDY IN RATS

Sponsor
Nippon Zeon Co Ltd
Corporate Business Development
Furukawa Sogo Bldg
6-1 Marunouchi 2-chome
Chiyoda-ku
Tokyo 100
JAPAN

Testing facility
Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Circulation list: **Sponsor (x2)**, QA, M Bannerman, C J Hardy, D W Coombs, G C Jackson, I Gilkison, R Conaghan.

18 March 1997

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

1. Introduction

1.1 The study described in this protocol is designed to investigate the acute inhalation toxicity of the test substance and, if appropriate, to determine the median lethal concentration LC_{50} (4 hour). The inhalation route is selected since this is a possible route of exposure in man.

The study is designed to be in compliance with EEC, OECD and US-EPA and J-MAFF test guidelines for acute inhalation studies.

The procedures to be used during the course of this study are those documented in the relevant Huntingdon Life Sciences Standard Operating Procedures Manual.

Throughout this protocol, the symbol '??' indicates that the relevant information is not available at present but will be detailed by protocol amendment.

1.2 Personnel:

Head, Inhalation Toxicology:	M Bannerman
Senior Inhalation Toxicologist:	C J Hardy
Study Director:	D W Coombs
Study Supervisor:	R Conaghan
Head, Aerosol Technology and Analysis:	I Gilkison
Veterinary Director:	D P Buist
Principal Pathologist:	C Gopinath
Monitoring Scientist:	Mr K Goto

In the absence of the Study Director, responsibility for the study will be assumed by the Senior Inhalation Toxicologist, Division of Toxicology.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

2. Test animals

2.1 Species and strain: Virgin young adult rats of Sprague Dawley origin.

2.2 Supplier: The rats will be ordered from Charles River Ltd. or an alternative supplier approved by the Veterinary Director, Huntingdon Life Sciences Ltd.

2.3 Number: Minimum of 10 male and 10 female rats for a limit test (1 control group and 1 test group each of 5 male and 5 female rats), increasing by 5 male rats and 5 female rats for each of any additional test groups if the study is extended to determine the LC_{50} (see Section 6.2).

2.4 Age: Approximately 7 weeks (males) or 8 weeks (females) when delivered to Huntingdon Life Sciences. Weight variations should not exceed ± 20% of the mean weight for each sex.

2.5 Justification of choice of species: The rat is a species recommended by all current test guidelines for this type of study.

In addition, we have many years experience in the use of the rat as an animal model in toxicology studies, and comprehensive background data.

2.6 Selection of animals: Any rat that is in poor condition or physically damaged will not be allocated to the study.

2.7 Allocation: The initial consignment of rats will be allocated to the following groups:

Group 1 (Control)
Group 2 (Test)

If the study is extended the additional rats will be allocated to the next logical test groups.

2.8 Identification: Each rat will be uniquely identified by a number tattooed on the ear pinnae and if necessary on the right hind foot to indicate 100's.

2.9 Acclimatisation: The rats will be held in our laboratories for at least 5 days prior to inhalation exposure.

2.10 Dead and moribund animals: Any rat that dies during the acclimatisation period will be replaced by a rat of the same sex and similar body weight.

Should any rat die or be sacrificed for humane reasons at any other time during the study it will be subjected to a complete macroscopic examination.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

3. Accommodation and Husbandry

3.1 Location of study: building Y13, rooms 7 and 8.

3.2 Cage type and size: the holding cages (size: 35 cm wide, 53 cm long and 25 cm high) are fabricated from stainless steel mesh and stainless steel sheet. The cages are suspended on racks. Plastic trays, lined with absorbent paper, will be placed below the cages to collect animal waste.

3.3 Number of animals per cage: 5 male or 5 female rats.

3.4 Cage labelling: each cage will bear a coloured label specifying the study number, the treatment group, the sex and the identification number of the rats allocated to the group.

3.5 Room temperature: the room temperature will be maintained at 22°C ± 3°C.

The temperature will be recorded continuously using a Kent Clearspan recorder.

3.6 Room relative humidity: the room relative humidity will be maintained at 55% ± 15% RH.

Relative humidity will be recorded continuously using a Kent Clearspan recorder.

3.7 Lighting: 12 hours light (08.00 - 20.00) and 12 hours dark controlled automatically.

3.8 Dry diet: SDS rat and mouse diet (RM1) will be available *ad. libitum* except during exposure.

3.9 Water: tap water from moulded polypropylene bottles will be available *ad.libitum* except during exposure. The bottles will be rinsed and refilled daily.

3.10 Analysis of food and water: there is no information available to indicate that any substance likely to influence the effect of the test compound can reasonably be expected to be present in the diet or drinking water.

Each batch of diet is analysed for nutrients and for specified substances and micro-organisms likely to be present in the diet and which, if in excess of specified amounts, might have an undesirable effect on the test system. Although occasional slight deviation may be permitted, batches of diet will conform with the acceptable standards agreed by the Study Director and Quality Assurance Department.

The water supplied to Huntingdon Life Sciences, by Anglian Water, is potable water for human consumption. Anglian Water takes its guidelines on water quality from the EEC directive relating to water for human consumption (80/778/EEC) and conforms to the United Kingdom Water Act 1989 and subsequent amendments.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

Results of routine physical and chemical examination of drinking water conducted by Anglian Water Services Ltd, are made available to Huntingdon Life Sciences as quarterly summaries.

The analytical data will be lodged in Huntingdon Life Sciences Archives.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

4. Test substance

4.1 Test substance

(a)	Chemical name:	1,2,3,4,4,5,5 octafluorocyclopentane
(b)	Common name	OFCPE
(c)	CAS number:	559-40-0
(d)	Presentation:	Low boiling point liquid
(e)	Received from:	??
(f)	On:	??
(g)	Batch No:	??
(h)	Purity:	99.7%
(i)	Expiry date	??

4.2 Storage: In the dark at room temperature, 4°C or -20°C and in the original container.

4.3 Archive sample: A small sample (1 to 2g) will be sealed in suitable container and stored in archives at an appropriate temperature.

4.4 Disposal: The surplus test material with the exception of the archive sample will be retained for 3 months following the study completion data. The surplus material will then be discarded or returned to the Sponsor.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

5. Inhalation Exposure

5.1 Duration: A single 4 hour continuous exposure using a snout-only exposure system.

The exposure will be timed for 4 hours following an equilibration period during which the concentration of the test substance is expected to attain 90% of its final concentration. With the system to be used this equilibration period will be 6 minutes.

5.2 Exposure chambers:

The snout-only exposure system is recommended for test substances which may be toxic by oral or dermal routes or are available only in limited quantities.

The snout-only chambers are of cylindrical form (10 cm diameter and 65 cm height) and made of aluminium alloy with a conformal resistant coating. The chambers have an internal volume of approximately 5 litres.

The rats are held for exposure in moulded polycarbonate tubes which are attached at evenly spaced ports in the cylindrical section of the chamber. The end of the tube attached to the chamber is tapered to allow the snout only to project into the exposure chamber. The other end of the tube is closed by a foamed plastic stopper. A push rod passes axially through the stopper and can be adjusted to maintain the rat in the forward position during the exposure.

5.3 Air flow: The chambers will be operated under dynamic air flow conditions. The flow of air to each chamber will be sufficient for at least 12 air changes per hour (approximately 2 litres per minute) and will be constant throughout the exposure period. The rate of airflow will be monitored continuously during exposure and will be recorded at the start of exposure and then at 30 minute intervals. The airflow rate will ensure that the test atmosphere contains at least 19% oxygen.

The test atmosphere enters at the top centre of the chamber and passes out through a port in the base of the chamber below the level of the rats. Each chamber is installed in a large fume cupboard exhausting to atmosphere through an absolute filter.

5.4 Test atmosphere temperature and relative humidity: The temperature in the exposure chamber will be monitored continuously during exposure and recorded at 30 minute intervals.

If possible the chamber humidity will be monitored continuously using an infra-red water vapour analyser or a Casella type T6900 relative humidity meter and recorded at 30 minute intervals. An accurate measurement may not be possible if the droplets or vapour of the test substance interfere with the normal operation of the instrument.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

5.5 Generation of the test atmosphere:

The test atmosphere will be prepared by metering liquid or gaseous test material into a mixing chamber where it will be mixed with the chamber air. Full details will be provided in the Final Report.

5.6 Atmosphere analysis: At least 5 samples of the chamber air will be removed in order to estimate the concentration of the test substance in the chamber air. The samples will be collected from the breathing zone of the rats. The air samples will be withdrawn through a suitable absorption trap or will be collected in a gas-tight syringe. If samples are withdrawn through an absorption trap the air sample volumes will be measured using a wet-type gas meter.

The amount of test substance collected will be determined by chemical analysis.

5.7 Control group: A similar exposure system will be used for the control group. Sections 5.2 to 5.4 will apply except that the rats will be exposed to clean air only. The duration of exposure will be 4 hours plus the equilibration time.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

6. Procedure

6.1 Generation Trials: A generation trial of up to 1 **hour duration** will be undertaken to establish the suitability of the test atmosphere generation system for use with the test substance. Particular attention will be given to stability of the concentration of the test substance. A small number of rats may be exposed to determine a suitable exposure concentration for the first test group.

6.2 Exposure: If a preliminary exposure has been conducted, a group of 5 male and 5 female rats will be exposed at a concentration consistent with the survival of some rats. Otherwise the first test group will be exposed at a concentration of 10% v/v (100,000 ppm)or to the maximum attainable concentration if this is lower.

If the first exposure indicates that the LC_{50} is less than 10000 ppm further groups of 5 male and 5 female rats will be exposed at different concentrations, spaced appropriately, to produce a range of mortality rates. A minimum of 2 and a maximum of 4 additional groups of rats will be used to derive a reliable LC_{50} for the test substance.

If there is a clear difference in the response of male and female rats, groups of rats of 1 sex only may be exposed in order to provide data to allow calculation of the LC_{50} for the sexes separately.

The control group (if applicable) will be subject to the same restraint and procedures except that no test substance will be introduced into the chamber.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

7. Observations

7.1 Clinical signs: All rats will be observed at hourly intervals or more frequently during exposure. Signs of reaction to exposure will be recorded in terms of time of onset, duration and intensity (where appropriate). The clinical signs will be recorded immediately post exposure and then at 1 hour and 2 hours post exposure. During the observation period a full clinical signs check will be performed daily in the morning. The rats will be checked for survival later in the day.

7.2 Deaths: The circumstances of any deaths will be recorded in detail, a full post mortem examination carried out and the macroscopic condition of all major internal organs noted.

7.3 Bodyweight: All rats will be weighed daily from the day of arrival at our laboratories, on Day 0 (immediately before exposure) and daily thereafter.

7.4 Food and water consumption (optional): The amounts of food and water consumed by each cage of rats will be measured daily

7.5 Observation period: The rats will be kept for an observation period of 14 days following exposure. This period may be extended at the discretion of the Study Director, if there is evidence of development of late toxicity or unusually slow recovery from the effects of exposure.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

8. Terminal Studies

8.1 Terminal kill: Rats surviving the observation period will be killed by intraperitoneal injection of pentobarbitone sodium and exsanguinated when clinically dead. A complete macroscopic examination of each rat will be performed.

8.2 Tissues will not be retained.

9. Evaluation of results

The effects of exposure to the test compound will be evaluated. If the study is extended, the median lethal concentration (LC_{50}) and confidence limits will be calculated using appropriate statistical procedures.

10. Reports

The report will contain a description of all methods used and the results of the study, including but not limited to the following:

The design, type, and dimensions of the exposure apparatus, and the method of housing the animals in the test chamber.

The methods for generating the test aerosol, source of air, aerosol conditioning and treatment of exhaust air.

The equipment used for measuring chamber air temperature, humidity, aerosol concentrations and particle size.

Clinical observations, necropsy findings and conclusions.

10.1 Draft report: One copy of a draft report will be sent to the Monitoring Scientist for comment.

10.2 Final report: Five copies (bound and or unbound) copies will be issued following an audit of the draft report by the Quality Assurance Department.

11. Archives

All raw data including the original protocol and report will be lodged in the Huntingdon Life Sciences Archives. The data will be retained for at least 5 years (10 years optional).

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

12. Good Laboratory Practice and Quality Assurance

This study will be conducted in compliance with the principles of Good Laboratory Practice as set forth in:

Good Laboratory Practice The United Kingdom Compliance, Department of Health and Social Security 1986 and subsequent revision, Department of Health, 1989.

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160 (FIFRA) or Part 792 (TSCA), Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984

The Huntingdon Life Sciences Quality Assurance Department will review the protocol for GLP completeness and audit the Final report for accuracy of reporting.

Certain studies such as that described in this protocol are conducted in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study is in progress, "processed-based" inspections of routine procedures will be made by the Quality Assurance Department. For the inspection of any given routine procedure, at least one study will be selected without bias. Critical phases of the study will be subjected to a study based inspection. The findings of these inspections will be reported promptly to the Study Director and to Management.

13. Records to be maintained

These are listed in Appendix 1.

14. Time plan

An outline time plan is shown in Appendix 2. The actual study dates will be advised by protocol amendment or a revised time plan when the study is initiated.

15. Protocol approval

A Protocol approval page is attached as the last page of the protocol.

16. Amendments

Amendments to this protocol may be made as the study progresses. No changes in the protocol, except where indicated, will be made without the request or consent of the Sponsor. Verbal requests for amendments will be honoured by Huntingdon Life Sciences but such requests should be confirmed in writing. All protocol amendments will be issued by the Study Director and signed by the Study Director and the Sponsor and or the Monitoring Scientist.

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

Appendix 1

Records to be maintained

1. Protocol and protocol addenda
2. Study schedule
3. Pre-initiation data
4. Technical personnel and signature list
5. Source, purchase order and delivery note of animals
6. Sex verification
7. Randomisation data - if applicable and allocation to groups
8. Animal identification
9. Diet batch number and analyses
10. Drinking water analyses
11. Room humidity and temperature
12. Animal husbandry
13. Analysis data
14. Clinical signs
15. Bodyweight data
16. Date and type of termination for each animal in the study
17. Necropsy findings and lung weight data/or other organs specified by the protocol
18. Histopathological data (if applicable)
19. Quality assurance

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

Appendix 2

Time plan

Arrival of animals:	??
Start of experimental work:	??
Start of main study	??
Completion of the study:	??
Submission of the Draft report:	??
Submission of the final report:	Within 28 days of receiving Sponsor comments

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Huntingdon Life Sciences Study Number: ZCE/13

Huntingdon Life Sciences

PROTOCOL APPROVAL

OFCPE

ACUTE INHALATION (4 HOUR) STUDY IN RATS

D W Coombs,
Study Director,
Huntingdon Life Sciences Ltd.

18 March 1997
Date

M Bannerman,
Management,
Huntingdon Life Sciences Ltd.

18 March 1997
Date

For Sponsor,
Nippon Zeon Co., Ltd.

Date

Monitoring Scientist
Mr Kuniaki Goto

25 March 1997
Date

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 1

Huntingdon Life Sciences

PROTOCOL AMENDMENT

OFCPE

ACUTE INHALATION (4 Hour) STUDY IN RATS

Study Director: D W Coombs B.Sc.

The signature of the Study Director authorises the implementation of this amendment to protocol.

AUTHORISATIONS TO PROTOCOL AMENDMENT NO. 1

For Huntingdon Life Sciences Ltd

Authorised by: _____ Date: 2 April 1997

D W Coombs
Study Director

For Nippon Zeon Co. Ltd

Approved by: Kuniaki Goto Date: 9 April 1997

Mr Kuniaki Goto
Monitoring Scientist

Distribution: Sponsor (2), QA, C J Hardy, M Bannerman, D W Coombs, R Conaghan, G Jackson, P Davies (2), I Gilkison (2).

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 1

Huntingdon Life Sciences

PROTOCOL AMENDMENT

OFCPE

ACUTE INHALATION (4 Hour) STUDY IN RATS

Study Director: D W Coombs B.Sc.

The signature of the Study Director authorises the implementation of this amendment to protocol.

AUTHORISATIONS TO PROTOCOL AMENDMENT NO. 1

For Huntingdon Life Sciences Ltd

Authorised by: _____ Date: 2 April 1997

D W Coombs
Study Director

For Nippon Zeon Co. Ltd

Approved by: Kuniaki Goto Date: 9 April 1997

Mr Kuniaki Goto
Monitoring Scientist

Distribution: Sponsor (2), QA, C J Hardy, M Bannerman, D W Coombs, R Conaghan, G Jackson, P Davies (2), I Gilkison (2).

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 1

Huntingdon Life Sciences

Reason for Amendment:

Addition of information relating to test substance.

Addition of time plan details

Modification of UK GLP statement

Amendment:

Page 2 of 15; section 1.1:

Delete the last sentence: 'Throughout this protocol, the symbol '??' indicates that the relevant information is not available at present but will be detailed by protocol amendment.'

Page 6 of 15; section 4.1:

Add: (e) Nippon Zeon Co. Ltd (via Huntingdon life sciences Co. Ltd, Japan)
(f) 25 March 1997
(g) Lot 9703-1
(i) Assumed stable for duration of the study

Page 9 of 15; section 6.2; Paragraph 2:

Change '...less than 10000 ppm...' to '...less than 100,000 ppm...'

Page 10 of 15; section 7.4:

'Food and water consumption (optional):...' Delete '..(optional)'

Page 12 of 15; section 12:

Change: 'Good Laboratory Practice The United Kingdom Compliance, Department of Health and Social Security 1986 and subsequent revision, Department of Health, 1989.'

To: 'Principles of Good Laboratory Practice as required by the United kingdom Good Laboratory Practice regulations 1997.'

Section 14:

Delete: ' The actual study dates will be advised by protocol amendment or a revised time plan when the study is initiated.'

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 1

Huntingdon Life Sciences

Page 14 of 15; Appendix 2:

Replace with:

Time plan

Arrival of animals:	2 April 1997
Start of experimental work (prelim.):	8 April 1997
Start of main study	11 April 1997
Completion of the study:	25 April 1997
Submission of the Draft report:	13 June 1997
Submission of the final report:	11 July 1997

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 2

Huntingdon Life Sciences

PROTOCOL AMENDMENT

OFCPE

ACUTE INHALATION (4 Hour) STUDY IN RATS

Study Director: D W Coombs B.Sc.

The signature of the Study Director authorises the implementation of this amendment to protocol.

AUTHORISATIONS TO PROTOCOL AMENDMENT NO. 2

For Huntingdon Life Sciences Ltd

Authorised by: _____ Date: 17 April 1997

D W Coombs
Study Director

For Nippon Zeon Co. Ltd

Approved by: Kuniaki Goto Date: 22 April 1997

Mr Kuniaki Goto
Monitoring Scientist

Distribution: Sponsor (2), QA, C J Hardy, M Bannerman, D W Coombs, R Conaghan, G Jackson, P Davies (2), I Gilkison (2).

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 2

Huntingdon Life Sciences

Reason for Amendment:

To correct the time plan.

Amendment:

Page 14 of 15; Appendix 2 and protocol amendment No. 1:

Replace with:

Time plan

Arrival of animals	(Batch 1):	2 April 1997
	(Batch 2):	18 April 1997
Start of experimental work (prelim.):		8 April 1997
Start of main study:		15 April 1997
Completion of the study:		09 May 1997
Submission of the Draft report:		27 June 1997
Submission of the final report:		25 July 1997

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 3

Huntingdon Life Sciences

PROTOCOL AMENDMENT

OFCPE

ACUTE INHALATION (4 Hour) STUDY IN RATS

Study Director: D W Coombs B.Sc.

The signature of the Study Director authorises the implementation of this amendment to protocol.

AUTHORISATIONS TO PROTOCOL AMENDMENT NO. 3

For Huntingdon Life Sciences Ltd

Authorised by: _____ Date: 3 June 1997

D W Coombs
Study Director

For Nippon Zeon Co. Ltd

Approved by: Kuniaki Goto Date: 9 June 1997

Mr Kuniaki Goto
Monitoring Scientist

Distribution: Sponsor (2), QA, C J Hardy, M Bannerman, D W Coombs, R Conaghan, G Jackson, P Davies (2), I Gilkison (2).

APPENDIX 3

(Protocol and Protocol Amendments - continued)

Study number: ZCE/13

Protocol amendment number: 3

Huntingdon Life Sciences

Reason for Amendment:

To alter the airflow and exposure chamber size for Groups 3, 4 and 5, due to relatively high toxicity and the necessity for adequate control at low concentrations.

To alter the time plan to accommodate the exposure of additional groups of rats.

Amendment:

Page 7 of 15; Section 5.2:

Paragraph 2 of this section is replaced with:

> The snout-only chambers used are of cylindrical form and made of aluminium alloy with a conformal resistant coating. The chambers used for Groups 1-3 (10 cm diameter and 65 cm height) have an internal volume of approximately 5 litres, the chamber used for Groups 4 and 5 (30 cm diameter and 45 cm height) has an internal volume of approximately 30 litres.

Page 7 of 15; Section 5.3:

The second sentence in Paragraph 1 is replaced with:

> The flow of air to each chamber will be sufficient for at least 12 air changes per hour (approximately 2 litres per minute for Groups 1 and 2, 5 litres per minute for Group 3 and 30 litres per minute for Groups 4 and 5) and will be constant throughout the exposure period.

Page 14 of 15; Appendix 2 and protocol amendment No. 2:

Replace with:

		Time plan
Arrival of animals	(Batch 1):	2 April 1997
	(Batch 2):	18 April 1997
	(Batch 3):	30 April 1997
Start of experimental work (prelim):		8 April 1997
Start of main study:		15 April 1997
Completion of the study:		22 May 1997
Submission of the draft report:		10 July 1997
Submission of the final report:		7 August 1997

CONFIDENTIAL BUSINESS INFORMATION

REPORT AMENDMENT

Amendment No.: 2

Huntingdon life sciences report No.: ZCE 13/972861

Date Final report issued: 16 January 1998

Date of Amendment: 17 July 1998

Study Director: D W Coombs

Amendment requested by: D W Coombs

Company: Nippon Zeon Co., Ltd., Japan

Authorisation signatures

Study Director: [signature]

Date: 17 July 1998

Quality Assurance: [signature]

Date: 17 July 1998

Details of Amendment:

Page 18: **Terminal studies**

Estimation of the LC_{50}(4-hour) for OFCPE

Text table;

	LC_{50}(4-hour) ppm	**95% CL (ppm)**
Males	490	175.5-805.0
Females	445	159.9-729.6
Combined	459	286.4-631.1

To read

	LC_{50}(4-hour) ppm	**95% CL (ppm)**
Males	445	159.9-729.6
Females	490	175.5-805.0
Combined	459	286.4-631.1

Reason for amendment:

Transposition error not detected at final author check